Science Library

APR 29 1946

Volume 36 *Number 4*

Phytopathology

AN INTERNATIONAL JOURNAL

April 1946

Official Organ of

The American Phytopathological Society

$6.25 per year, Canada $6.00 per year, United States and dependencies $6.50 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter.

TABLE OF CONTENTS

Leon Hatchig Leonian, 1888–1945 ..... C. R. Orton 241

Diplodia rot of onions.
Glen B. Ramsey, Barbara C. Heiberg, and James S. Wiant 245

Influence of time, rate, and depth of seeding on the incidence of root rot in wheat ..... F. J. Greaney 252

Exploratory experiments with the big-vein disease of lettuce.
Dean E. Pryor 264

Peach blotch ..... R. S. Willison 273

Growth and overwintering of Xanthomonas vesicatoria in association with wheat roots ..... Stephen Diachun and W. D. Valleau 277

Failure of bud and graft unions of rose induced by Chalaropsis thielavioides ..... Kenneth F. Baker and H. Earl Thomas 281

Reaction of onion varieties to yellow-dwarf virus and to three similar viruses isolated from shallot, garlic, and narcissus.
Philip Brierley and Floyd F. Smith 292

Influence of nitrogen nutrition on susceptibility of onions to yellow-dwarf virus ..... Philip Brierley and Neil W. Stuart 297

The relation of soil temperature to the development of Armillaria root rot ..... Donald E. Bliss 302

Phytopathological Notes 319

Bud blight of soybean caused by the tobacco ring-spot virus.
William B. Allington

Two aids for the study of potato-late-blight epidemiology.
W. D. Thomas, Jr.

A new bean mosaic in Idaho.
Leslie L. Dean and C. W. Hungerford

PHYTOPATHOLOGY

An International Journal
Official Organ of The American Phytopathological Society

EDITORS

HELEN HART, Editor-in-Chief, University Farm, St. Paul 8, Minnesota.
W. H. BURKHOLDER, Cornell University, Ithaca, New York.
A. J. RIKER, University of Wisconsin, Madison 3, Wisconsin.
W. J. ZAUMEYER, Bureau of Plant Industry Station, Beltsville, Maryland.

ASSOCIATE EDITORS

E. J. ANDERSON, State College of Washington, Pullman, Washington.
L. M. BLACK, Rockefeller Institute for Medical Research, Princeton, New Jersey.
A. A. DUNLAP, Agricultural Experiment Station, College Station, Texas.
M. W. GARDNER, University of California, Berkeley 4, California.
J. M. HAMILTON, N. Y. Agricultural Experiment Station, Geneva, New York.
F. L. HOWARD, Rhode Island State College, Kingston, Rhode Island.
W. G. HOYMAN, North Dakota State College, Fargo, North Dakota.
G. C. KENT, Iowa State College, Ames, Iowa.
H. N. RACICOT, Experimental Farm, Ottawa, Ontario, Canada.
L. R. TEHON, State Natural History Survey, Urbana, Illinois.
C. M. TUCKER, University of Missouri, Columbia, Missouri.
W. W. WAGENER, 720 Appraisers Building, San Francisco 11, California.

BUSINESS MANAGEMENT

R. M. CALDWELL, Business Manager, Purdue University, West Layfette, Indiana.
PAUL E. TILFORD, Advertising Manager, Agricultural Experiment Station, Wooster, Ohio.

NOTICES

All communications regarding subscriptions and back numbers should be sent to R. M. CALDWELL, Business Manager, A.E.S. Bldg., Purdue University, Layfette, Indiana.

Subscription price. $6.00 per year United States and dependencies; Canada, $6.25; other countries $6.50; single numbers of the current volume, 60 cents each. The journal has been issued monthly beginning with Volume VIII, January, 1918. Prior to that it was issued bimonthly.

Separate Numbers may be purchased from broken volumes, and stocks available are as follows: Vol. 1, No. 1, index; Vol. 2, Nos. 1, 5, index; Vol. 3, No. 5; Vol. 6, index; Vol. 8, Nos. 2, 3, 5, 6, 7, and 9 through 12; Vol. 9, Nos. 2 through 12, index; Vol. 10, Nos. 2 through 12. Separate numbers of volumes 1 through 10 are sold at $2.00 per copy, those of volumes 11 through 35 at $1.00 a copy. Complete, unbound volumes 11 through 35 are available at $8.00 a volume.

Thirty-Year Index (to Vols. 1–30, 1911–1940) bound. $5.50, United States. $6.00, elsewhere.

Advertisements. Rates may be secured from the Advertising Manager. No indorsement of any statement of claims made in advertisement is assumed by this journal or by The American Phytopathological Society.

Lost Numbers. Claims for copies (domestic subscriptions) lost in the mails must be received within 30 days from the date of issue (Jan. 15 and monthly thereafter); otherwise the usual charge of 60¢ per copy will be made for replacements. Deliveries overseas are not guaranteed for the duration of the war and foreign mailings are made at the subscriber's risk.

Restoration to Active Membership. Restoration requires no special action. No penality is imposed and no back dues are required. Application for reinstatement accompanied by check or money order for the current dues ($5) should be sent to the secretary. All issues of Phytopathology for the year will be supplied. Back volumes may be purchased, as far as available, at $8 each.

Separates. No gratis copies supplied. A printed schedule of prices is submitted with the proof, and authors may secure separates by placing an order on the blanks provided when proof is returned.

Manuscript may be sent to the nearest member of the editorial board. Clearness, brevity, and conciseness are essential. In form and style, such as punctuation, spelling and use of italic, manuscript should conform to the best usage in recent numbers of this journal. Manuscripts should be typed on one side of the paper, double spaced, and sent unfolded. All papers longer than four printed pages should include a summary. The name and address of the institution at which the work was done should be included at the end of the summary. All footnotes should be numbered consecutively with arabic numerals except in tables, where small letters should be used. Each table should have a heading stating clearly and concisely its contents. References to literature should be made as footnotes only when five or fewer citations are given. The simplified method of citing literature used in recent numbers of this journal should be followed. References in "Literature Cited" should be listed alphabetically and numbered consecutively.

Illustrations necessarily must be limited in number. Photographs, to reproduce satisfactorily, must be of best quality. Authors who desire unusual numbers of illustrations or special types of illustrations will be asked to bear part of the cost, i.e. in excess of $12.50, the approximate cost of 2 full-page cuts. All illustrations are reproduced on the same grade of paper and will be run as text figures unless authors specify otherwise. Since plates are printed on the same kind and quality of paper, they offer no advantage over full-page figures. There is, therefore, nothing to be gained through publication of illustrations as plates rather than as figures. Individual illustrations within a figure—or plate—should be designated by letters to avoid confusion with text figures, designated by arabic numerals. Full-page figures—or plates—should be arranged to conform to journal page size and shape ($4\frac{1}{2}'' \times 7''$) after making due allowance for reductions. Specific reference to each illustration must be made in the text. Each illustration must be accompanied by an adequate legend, the legend to be typed on separate paper and not on the illustration.

The responsibility for statements, whether of fact or opinion, rests entirely with the writers thereof.

The American Phytopathological Society

FOUNDED 1909. INCORPORATED 1915

The A. P. S. Council for 1945

President—H. B. Humphrey, Box 14, Cosmos Club, Washington 5, D. C.

Vice-President—J. H. Graigie, Dominion Rust Research Laboratory, Univ. of Manitoba, Winnipeg, Manitoba, Canada

Secretary—E. M. Johnson, Agricultural Experiment Station, Lexington 29, Kentucky

Treasurer—R. M. Caldwell, Dept. Botany and Plant Pathology, Purdue University, Layfayette, Indiana

Editor-in-Chief—Helen Hart, University Farm, St. Paul 8, Minnesota

Councilors:

C. H. Arndt, Agricultural Experiment Station, Clemson College, South Carolina

R. J. Haskell, Extension Service, U. S. Dept. Agr., Washington 25, D. C.

M. C. Richards, University of New Hampshire, Durham, N. H.

R. S. Kirby, Pa. State College, State College, Pennsylvania

R. W. Goss, Dept. Plant Pathology, College of Agriculture, Lincoln 1, Nebraska

L. D. Leach, Div. Plant Pathology, University Farm, Davis, California

J. J. Christensen, Div. Plant Pathology, University Farm, St. Paul 8, Minnesota

Sustaining Contributors:

Rohm and Haas, Washington Square, Philadelphia 5, Pennsylvania

E. I. du Pont de Nemours & Company, Wilmington 98, Delaware

Freeport Sulphur Company, New Orleans 5, Louisiana

Tennessee Corporation, 619–27 Grant Building, Atlanta 1, Georgia.

LEON HATCHIG LEONIAN

1888–1945

C. R. Orton

Dr. Leon Hatchig Leonian was born at Van, Armenia, February 27, 1888, and received his secondary education there. He emigrated to the United States at the age of 20, where he worked for a few years in New York and in Detroit.

He was graduated from the University of Kentucky with the B.S. degree in 1916 and from the University of Michigan with the degree of M.S. in 1917. Following a year as Assistant Research Horticulturist at Clemson College and a period as Assistant Professor of Botany and Plant Pathology in the New Mexico State College and Experiment Station, he returned to the University of Michigan. There he studied Mycology under Kaufmann and received the Ph.D. degree in 1922. That year he was appointed Assistant Plant Pathologist in the College of Agriculture and Experiment Station at West Virginia University. Here he became Professor of Mycology and Mycologist in the Experiment Station in 1936, the position he held to the time of his death, June 7, 1945.

Professor Leonian's early work was devoted to the study and control of plant diseases, particularly those caused by the downy mildews and by the Fusaria. His training and his experiences with the behavior of fungi led him into more detailed studies of the physiology of fungi, studies which became his major research program during his last fifteen years. His work on the influence of growth and sexuality factors in fungi and on growth factors for bacteria was productive of a long series of publications in ranking scientific journals.

Dr. Leonian was highly individualistic, always interested in living organisms, their habits of development and reproduction, their growth and metabolism. He was never interested in dead specimens. He abhorred the herbarium. To him taxonomy by the accustomed method of studying herbarium specimens was a great waste of time. His ultimate aim was to discover as many potentialities as possible of those living organisms in which he was particularly interested. His later association with Dr. V. G. Lilly enabled him to delve deeper into the field of mineral and vitamin metabolism of several groups of lower organisms—work which was productive of several new contributions to science.

As an avocation he became interested in the breeding of delphiniums, day lillies, and oriental poppies, achieving outstanding results from hybridization. Seeds from his "Lyondel Gardens" justly received international acclaim. With his ability as a speaker and as a flower breeder, he was much in demand as a speaker at garden club conventions in a several-state area. For many years he edited the Delphinium Year Book and in 1935 published "How to Grow Delphiniums" in book form.

LEON HATCHIG LEONIAN
1888–1945

As a teacher his knowledge, his ready wit, and his sympathetic understanding of their problems made him a favorite with his students. In tribute to his interest in the welfare of students, his friends and associates have established a Student Loan Fund in his memory.

A large host of friends and associates will remember for their life-time his frank ways and genial smile.

PUBLICATIONS OF LEON HATCHIG LEONIAN

Fusarium wilt of chile pepper. New Mexico Agr. Exp. Sta. Bul. 121. 1919.
Studies on the Valsa apple canker in New Mexico. Phytopath. **11**: 237–242. 1921.
Stem and fruit blight of pepper caused by *Phytophthora capsici* sp. nov. Phytopath. **12**: 401–408. 1922.
The physiology of perithecial and pycnidial formation of *Valsa leucostoma*. Phytopath. **13**: 257–272. 1923.
A study of factors promoting pycnidium-formation in some Sphaeropsidales. Amer. Jour. Bot. **11**: 19–50. 1924.
Physiological studies on the genus *Phytophthora*. Amer. Jour. Bot. **12**: 444–498. 1925.
The morphology and the pathogenicity of some Phytophthora mutations. Phytopath. **16**: 723–730. 1926.
The effect of different hosts upon the sporangia of some Phytophthoras. Phytopath. **17**: 483–490. 1927.
Studies on the variability and dissociation in the genus *Fusarium*. Phytopath. **19**: 753–868. 1929.
Comparative value of the size of Phytophthora sporangia obtained under standardized conditions. Jour. Agr. Res. [U.S.] **39**: 293–311. 1929. (With H. L. GREER.)
Differential growth of Phytophthoras under the action of malachite green. Amer. Jour. Bot. **17**: 671–677. 1930.
Attempt to induce mixochimaera in *Fusarium moniliforme*. Phytopath. **20**: 895–901. 1930.
Heterothallism in *Phytophthora*. Phytopath. **21**: 941–955. 1931.
The effect of position of the inoculum upon the growth of some *Trichophytons* in the presence of dyes. Arch. Derm. and Syph. **25**: 1016–1020. 1932.
The pathogenicity and the variability of *Fusarium moniliforme* from corn. W. Va. Agr. Exp. Station Bul. 248. 1932.
Identification of *Phytophthora* species. W. Va. Agr. Exp. Sta. Bul. 262. 1934.
The effect of auxins upon *Phytophthora cactorum*. Jour. Agr. Res. [U.S.] **51**:277–286. 1935.
Control of sexual reproduction in *Phytophthora cactorum*. Amer. Jour. Bot. **23**: 188–190. 1936.
The effect of auxins from some green algae upon *Phytophthora cactorum*. Bot. Gaz. **97**: 854–859. 1936.
Is heteroauxin a growth-promoting substance? Amer. Jour. Bot. **24**: 135–139. 1937. (With V. G. LILLY.)
Partial purification of a vitamin-like substance which stimulates sexual reproduction in certain fungi. Amer. Jour. Bot. **24**: 700–702. 1937. (With V. G. LILLY.)
Studies on the nutrition of fungi. I. Thiamin, its constituents and the source of nitrogen. Phytopath. **28**: 531–548. 1938. (With V. G. LILLY.)
Studies on the nutrition of fungi. II. Effect of the inoculum on the growth of the colony. Phytopath. **29**: 592–596. 1939. (With V. G. LILLY.)
Vitamin B_1 in soil. Science **89**: 292–293. 1939. (With V. G. LILLY.)
Studies on the nutrition of fungi. III. Auxithals synthesized by some filamentous fungi. Plant Physiology **15**: 515–525. 1940. (With V. G. LILLY.)
Studies on the nutrition of fungi. IV. Factors influencing the growth of some thiamin-requiring fungi. Amer. Jour. Bot. **27**: 18–26. 1940. (With V. G. LILLY.)
Studies on the nutrition of fungi. V. Factors affecting zygospore formation in *Phycomyces blakesleeanus*. Amer. Jour. Bot. **27**: 670–675. 1940. (With V. G. LILLY.)
The growth rate of some fungi in the presence of cocarboxylase, and the moieties of thiamin. Proc. W. Va. Acad. Sci. **14**: 44–49. 1940. (W. Va. Univ. Bul. Ser. 41, No. 4–II. 1940.) (With V. G. LILLY.)
Factors affecting the yield of some thiamin-requiring fungi. Proc. Third International Congress for Microbiology, New York, 1939. pp. 496–498. 1940.
Some factors affecting the dry weight of *Chlorella vulgaris*. Amer. Jour. Bot. **28**: 569–572. 1941. (With V. G. LILLY.)

The effect of vitamins on ten strains of *Saccharomyces cerevisiae*. Amer. Jour. Bot. **29**: 459–464. 1942. (With V. G. LILLY.)
Vitamin synthesis by a yeast converted from a heterotrophic to an autotrophic habit. Science **95**: 658. 1942. (With V. G. LILLY.)
The "unknown factor" in the growth of *Saccharomyces cerevisiae*. Jour. Bact. **45**: 191–192. 1943. (With V. G. LILLY.)
Induced autotrophism in yeast. Jour. Bact. **45**: 329–339. 1943. (With V. G. LILLY.)
The effect of pH on the availability of *p*-aminobenzoic acid to *Neurospora crassa*. Science **99**: 18–19. 1944. (With O. WYSS and V. G. LILLY.)
The anti-biotin effect of desthiobiotin. Science **99**: 205–206. 1944. (With V. G. LILLY.)
Nitrogen metabolism in *Saccharomyces cerevisiae*. Proc. W. Va. Acad. Sci. **16**: 60–70. 1944. (W. Va. Univ. Bul. Ser. 44, No. 9–I. 1944.) (With V. G. LILLY.)
Conversion of desthiobiotin into biotin or biotin-like substances by some microorganisms. Jour. Bact. **49**: 291–297. 1945. (With V. G. LILLY.)
A quantitative comparison of biotin and desthiobiotin for the growth of certain microorganisms. Proc. W. Va. Acad. Sci. **17**: 55–61. (W. Va. Univ. Bul. Ser. 45, No. 10–I. 1945.) (With V. G. LILLY.)
The comparative value of different test organisms in the microbiological assay of B vitamins. W. Va. Agr. Exp. Sta. Bul. 319. 1945. (With V. G. LILLY.)
The interrelationship of iron and certain accessory factors in the growth of Rhizobium trifolii, strain 205. Jour. Bact. **50**: 383–395. 1945. (With V. G. LILLY.)

DIPLODIA ROT OF ONIONS

GLEN B. RAMSEY,[1] BARBARA C. HEIBERG,[2] AND JAMES S. WIANT[3]

(Accepted for publication October 10, 1945)

A dry or leathery rot of Texas-grown Crystal Wax onions caused by a species of *Diplodia* was first found on the Chicago market in June, 1938 (5), and on the New York, N. Y., market during the following season. The disease has been observed from time to time each year since then and was unusually prevalent on both markets during 1944. The decay has been found only on Texas-grown onions and only on the white varieties. Colored onions shipped from Texas during June do not show the disease. Although as high as 25 per cent of some lots of white onions have been affected, ordinarily the losses from Diplodia rot are not of great economic importance. However, the black discolorations produced are considered serious blemishes on white onions and the market value is reduced out of proportion to the actual decay present.

On market onions the characteristic symptom of this disease is silvery-gray to black discoloration of the outer dry scales about the upper half of the bulbs (Fig. 1, A, B, C). Occasionally the dry scales all over the onion are discolored (Fig. 1, F, G). In many onions dead and dying portions of the outer fleshy scales are also invaded and become black and leathery (Fig. 1, B, D) but the succulent living part of these scales is not invaded. In the more advanced stages black pycnidia singly or in groups of two or three are visible on dead scales (Fig. 1, C, F, G). Much of the mycelium that causes the dark discoloration is on the surface of the outer dry scale and between this scale and the first fleshy scale (Fig. 2, A, B). No decay of the internal fleshy scales has ever been observed.

Isolations from the discolored dry outer scales and from the dying tips of the first fleshy scales at the neck have consistently yielded a species of *Diplodia*. The spores produced are for the most part one-celled and hyaline (Fig. 2, C), but with age a high proportion of them become brown, striated, and 2-celled (Fig. 2, D). Spores obtained from pycnidia on onions and those produced in pure cultures usually range from 10.2 to 17.7 × 20.0 to 28.4 μ, averaging 12.8 × 23.6 μ. Neither in pure culture nor in diseased onion tissue has a perfect stage been observed.

On potato-dextrose-agar plates the minimum temperature for growth of the fungus was 50° F.; the optimum 85°; and the maximum 104°.

In plate cultures the onion *Diplodia* was compared with 12 other isolates of *Diplodia* from six different hosts, namely, avocado, cocoanut, orange, peanut, sweet potato, and watermelon. Although variations in character of growth and in spore measurements were observed, in no instance did they

[1] Senior Pathologist, [2] Scientific Aide, [3] Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

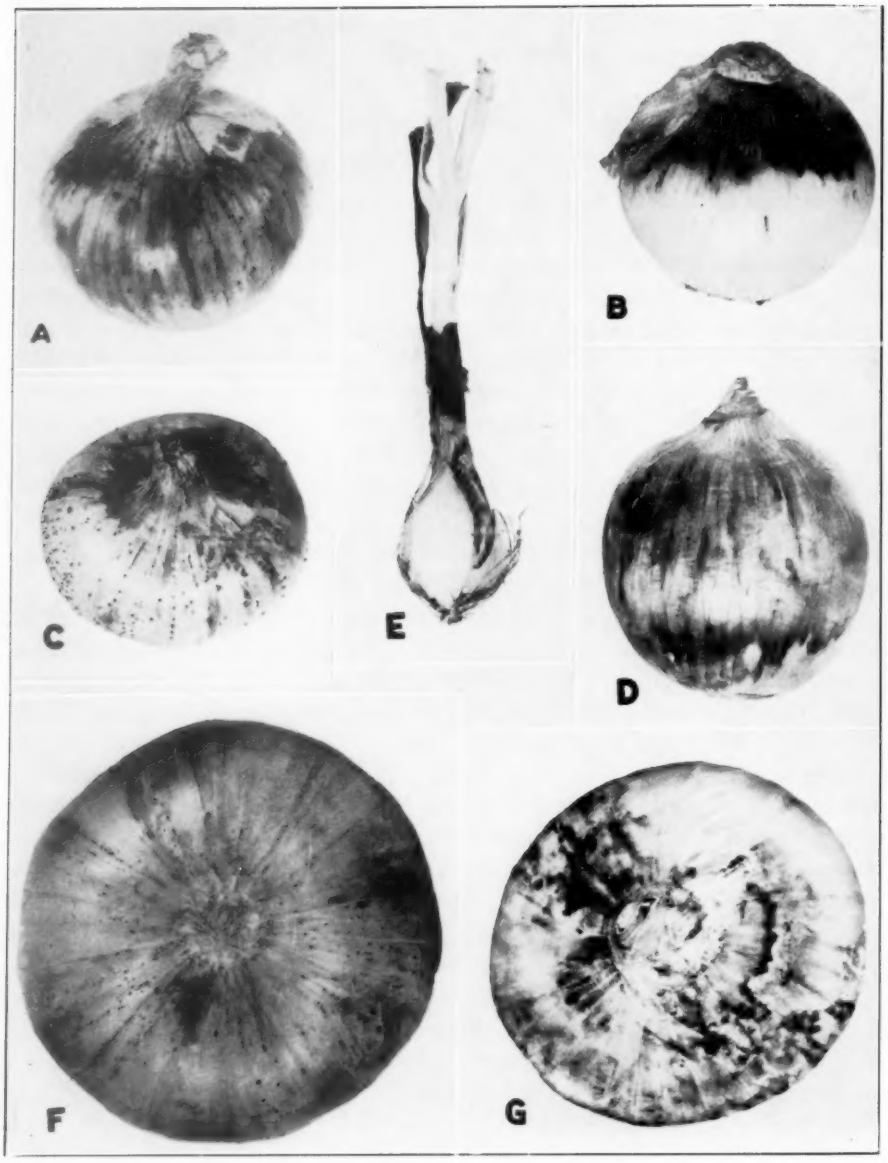


FIG. 1. Diplodia rot of onion. A, B, C. Naturally infected white onions with typical discoloration and decay as noted on the market. D. Leathery type of decay on dying outer fleshy scales of naturally infected white onion. E. Discoloration of dry scales at neck of onion seedling resulting from artificial inoculation. F, G. Advanced stages of discoloration of outer scales of naturally infected white onion. Pycnidia are abundant in C, F, and G.

appear to be of sufficient importance to indicate that distinct species were involved.

The onion *Diplodia* has been proven pathogenic to sweet potato, apple, and orange by means of inoculation experiments. Apples inoculated through a side wound developed an extensive brownish-black lesion within a week and ultimately the whole fruit was converted into a black, moderately firm mummy. Pycnidia were formed in the peel and the internal tissues were full of large hyphae typical for *Diplodia*. Stem-end inoculations of oranges with the onion *Diplodia* produced typical stem-end-rot symptoms identical with those produced by *D. natalensis* from oranges. In other cross-inoculation studies isolates of *Diplodia* from onion, watermelon, sweet potatoes, and

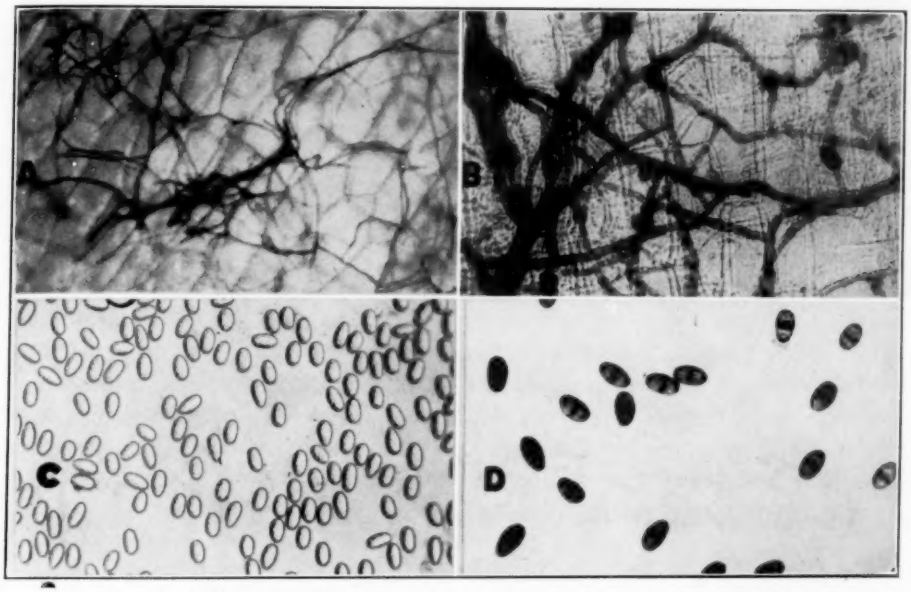


FIG. 2. *Diplodia natalensis* from onion. A and B. Hyphae on and between outer scales of white onion bulbs. 100× and 215×, respectively. C. Young 1-cell hyaline spores. 215×. D. Brown, striated, 2-cell spores. 215×.

oranges proved pathogenic when inoculated in sweet potatoes. A dark brown, fairly firm, moist decay with a sharp line of demarcation between the diseased and healthy tissue, characteristic of Java Black Rot in sweet potatoes, was produced in each instance.

These results are in agreement with those of others (1, 2, 6, 7) who have shown that strains of *Diplodia* from a wide variety of crop plants are similar morphologically and produce similar symptoms when cross-inoculated on a number of different host plants.

On the basis of the present studies the writers consider the onion *Diplodia* to be a strain of *Diplodia natalensis* Pole-Evans.

The invasion of onion by *Diplodia* always appears to be limited to the dead outer scales and the dying fleshy scales at the neck and down the side

of the onion, thus indicating that the fungus is saprophytic or only weakly parasitic to onions. Some growth of the fungus was obtained by inoculating the dry scales of a young onion plant just above the bulb and holding it in a moist chamber at 75° F. for ten days (Fig. 1, E). The only growth ever obtained in the fleshy scales was in white peeled onions that had been dipped in boiling water long enough to kill the tissues of the outer scales. All attempts to produce decay by inoculating fleshy succulent scales of white, yellow, and red onions have failed. This is in line with observations of this disease on market onions.

In view of the fact that growth of this *Diplodia* seems limited to white

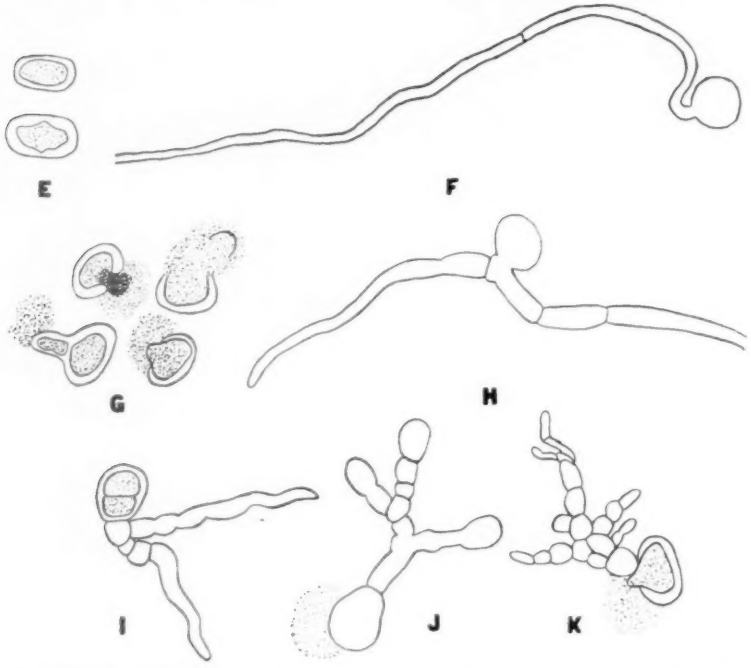


FIG. 3. *Diplodia natalensis* from onion. Camera lucida drawings (375 ×). E. Spores plasmolized by juice of fleshy scales. F. Normal germination of spore in water. G. Abnormal germination in a water extract of dry colored onion scales, with bursting of spores and extrusion of contents. H. Spore germinated in presence of dry white onion scales. I, J, and K. Abnormal germination in water extracts of yellow, brown, and red scales, respectively.

onions it was decided to check the reaction of this fungus to the color pigments in yellow, brown, and red onions to determine whether they were responsible for the apparent immunity of colored onions. To do this a suspension of Diplodia spores in sterile water was made and drops of the suspension were placed on sterile cover slips. Approximately 4 sq.mm. of colored or white onion scale was placed in these drops and the slips were then inverted over Van Tieghem cells. Drops of the spore suspension placed over Van Tieghem cells without the addition of onion scale were used as controls. All cells were allowed to stand from 18 to 24 hours at room temperature and

then counts of germinated spores were made. From counts of over 1,000 spores for each type of scale used, it was found that 92 per cent of the spores germinated in the control cells, 94 per cent in the presence of the white scale material (Crystal Wax), 66 per cent with the yellow scale (Yellow Bermuda), and 60 per cent with the red scale (California Red). The germ tubes produced by spores in the presence of the white scale tissue were normal (Fig. 3, H), whereas those produced in the presence of yellow and red scale tissue were abnormal. There were no normal germ tubes in the presence of any colored scale material and about half of the ungerminated spores burst and extruded their cytoplasm (Fig. 3, G). The germ tubes that were developed were shorter, thicker, more branched, and more septate than normal (Fig. 3, I, J, K). The germ tubes that developed in the controls and in the presence of white scale tissue were long and slender with few cross walls and little branching (Fig. 3, F, H). The reaction of this fungus to the pigmented onion scales is very similar to that of *Colletotrichum circinans* (Berk.) Voglino as reported by Walker and others (8, 9, 10, 11).

The effect of water extracts of pigmented onion scales on spore germination of *Diplodia* was tested in many experiments. Except for slight variations, the results obtained were usually similar to those just reported. However, it was noted that the degree of pigmentation (yellow, brown, or red) influenced the character and amount of germination. The more concentrated the pigment, the less the germination and the more abnormal the germ tubes produced. The water extract of dark scales of Australian Brown onions inhibited spore germination to a greater degree than the colored scales of any variety tested. It was also noted that the spores farthest from the scale particle in the hanging drop had more nearly normal germination and growth than those nearest it. The indications are, therefore, that some chemical substance associated with the pigment in the dry outer scales is the principal inhibiting agent for *Diplodia*. In view of the work of Link *et al.* (3, 4), it is assumed that this chemical is probably protocatechuic acid.

Since the internal fleshy bulb scales of neither the white nor colored varieties of onion are invaded by *Diplodia* it seemed desirable to test the effects of the presence of bits of fleshy scale of white and colored onions on spore germination. This was done by placing a 4-sq.mm. piece of fleshy scale in a hanging-drop water suspension of spores in Van Tieghem cells, and also by expressing the juice, diluting it in a spore suspension in sterile water, and observing in hanging drops. Although the spores germinated in the dilute juice exuding from bits of fleshy scales and in dilute juice extract of all varieties tested in the hanging drops in Van Tieghem cells, at higher concentrations of juice, germination was abnormal or totally inhibited and many of the spores were plasmolized (Fig. 3, E). In an experiment in which the freshly extracted juice from fleshy scales was used in a one to one dilution with a water suspension of spores 2 per cent germinated, whereas at a 1 to 5 dilution 7 per cent germinated; at 1 to 10, 10 per cent, and at 1 to 20, 62 per cent. In the controls 61 per cent germinated. The inability of

Diplodia spores to germinate normally in onion juice probably accounts for the fact that no invasion of fleshy wounds has been found in onions on the market.

In an attempt to determine whether or not the vapors from fleshy scales would inhibit growth of *Diplodia,* Petri-dish cultures seeded with spores were turned upside down and into the lid was introduced the freshly expressed concentrated juice of a red onion. Practically all of the spores germinated and a normal growth of *Diplodia* was produced within 24 hours, thus indicating that the growth of *Diplodia* is not inhibited by volatile substances given off by the juice of fleshy scales.

The hydrogen-ion concentration of water extracts from white, yellow, and red dry scales and of the fleshy inner scales of three varieties of onion (Crystal Wax, Yellow Bermuda, and California Red) was determined in order to ascertain whether acidity might be a factor in limiting the invasion of onion tissue by *Diplodia.* The dry outer scales of each variety listed had a pH value of 4.4 to 4.5. The juice of the fleshy scales was practically identical for all three varieties, ranging from pH 5.7 to 5.9. Cultures of the onion *Diplodia* grew well on potato-dextrose agar adjusted to pH 4.4 and 5.5 but developed slightly more slowly than on the same medium adjusted to pH 6.8. Growth on the medium at pH 4.4 was retarded only slightly more than at pH 5.5. Since there was little variation in the acidity of the dry scales or of the fleshy scales in the white susceptible variety in comparison with colored immune varieties of onion, and since the fungus grew well within the acid range found in these scales, these data indicate that the acidity of onion juice is not the primary factor in preventing *Diplodia* from becoming an active decay-producing organism in onions.

SUMMARY

A moderately serious market disease of Texas-grown white-skin Crystal Wax onions caused by *Diplodia natalensis* Pole-Evans is described for the first time. Colored-skin varieties of onions from Texas were not affected.

Only the dead outer scales and the dying parts of the outer fleshy scales of white onions are invaded. No decay of living fleshy scales of bulbs has been observed and all attempts at inoculation of such tissues have failed.

A chemical (probably protocatechuic acid) associated with the pigments in water extracts of the dry outer scales of colored varieties of onions proved toxic to the onion-Diplodia spores. Dry scales of white onions do not carry this toxic agent in sufficient quantity to interfere with the germination of the spores.

Determination of the pH of both the dry outer scales and the fleshy scales of white and of colored varieties of onion shows that acidity of these tissues is not the primary factor in limiting the pathogenicity of the onion *Diplodia* to white varieties.

The studies here reported and the market observations made during the past seven years indicate that although *Diplodia natalensis* is not strongly

pathogenic to onions, it may cause a great reduction in market value by producing slight decay and by blemishing the southern white onion crop.

U. S. DEPARTMENT OF AGRICULTURE,
CHICAGO, ILLINOIS.

LITERATURE CITED

1. EDDINS, A. H. A new diplodia ear rot of corn. Phytopath. **20**: 733–742. 1930.
2. ———, and R. K. VOORHEES. *Physalospora zeicola* on corn and its taxonomic host relationships. Phytopath. **23**: 63–72. 1933.
3. LINK, K. P., H. R. ANGELL, and J. C. WALKER. The isolation of protocatechuic acid from pigmented onion scales and its significance in relation to disease resistance in onions. Jour. Biol. Chem. **81**: 369–375. 1929.
4. ———, ALLAN D. DICKSON, and J. C. WALKER. Further observations on the occurrence of protocatechuic acid in pigmented onion scales and its relation to disease resistance in the onion. Jour. Biol. Chem. **84**: 719–725. 1929.
5. RAMSEY, G. B. Fruit and vegetable diseases on the Chicago market in 1938. U. S. Dept. Agr. Plant Dis. Reptr. Suppl. **114**: 32. 1939.
6. STEVENS, NEIL E. Two species of *Physalospora* on citrus and other hosts. Mycologia **18**: 206–217. 1926.
7. ———. Host relations in species of *Diplodia* and similar genera. Mycologia **33**: 69–73. 1941.
8. WALKER, J. C. Disease resistance to onion smudge. Jour. Agr. Res. [U.S.] **24**: 1019–1040. 1923.
9. ———. Further studies on the relation of onion scale pigmentation to disease resistance. Jour. Agr. Res. [U.S.] **29**: 507–514. 1924.
10. ———, CARL C. LINDEGREN, and FREDA M. BACHMANN. Further studies on the toxicity of onion juice extracted from succulent onion scales. Jour. Agr. Res. [U.S.] **30**: 175–187. 1925.
11. ———, K. P. LINK, and H. R. ANGELL. Chemical aspects of disease resistance in the onion. Proc. Nat. Acad. Sci. (Washington) **15**: 845–850. 1929.

INFLUENCE OF TIME, RATE, AND DEPTH OF SEEDING ON THE INCIDENCE OF ROOT ROT IN WHEAT[1]

F. J. GREANEY[2]

(Accepted for publication October 20, 1945)

INTRODUCTION

Common root rot is by far the most widespread and destructive root disease of wheat in Manitoba. It attacks both seedlings and mature plants. Although *Helminthosporium sativum* P. K. and B. and certain species of *Fusarium* are considered the primary cause of common root rot, other soil-borne fungi are constantly associated with the disease in this Province. Furthermore, drought, poor nutrition, and other environmental factors are almost invariably associated with the disease in the field.

The loss to the wheat crop of Manitoba from common root rot is substantial. For the 10-year period 1928–1937, the average annual loss to this Province was estimated to be not less than 1,909,000 bushels, or an annual cash loss of $1,336,000.00 for that period (2). This estimate, however, was based on the results obtained in experimental plots at Winnipeg and, when made, was recognized to be extremely conservative, as is now evident. On the basis of intensive systematic field surveys, Machacek (14) estimated that, for the 3 years, 1939 to 1942, the annual reduction in yield of wheat in Manitoba from common root rot was 12.1 per cent. This represented an average yearly monetary loss of $3,827,000.00.

The simplest and most effective method of reducing the losses caused by common root rot is by the use of varieties in which resistance to the disease has been combined with other desirable qualities by the process of hybridization and selection. Although certain varieties of wheat are known to be much more resistant than others, the problem of breeding varieties possessing high resistance to root rot is a difficult one, and there is the possibility that varieties wholly resistant under all environmental conditions may not be obtained for a considerable time. In the meantime, the control of the disease must depend upon other preventive measures such as crop rotation, manurial treatments, and cultural practices.

The present paper is concerned with the influence of certain cultural practices on the control of root rot of wheat, and reports the results of field experiments, carried out from 1936 to 1944, to determine the relation between the date, rate, and depth of seeding of spring wheat and the incidence of root rot caused by *Helminthosporium sativum* and *Fusarium spp.* in seedling and adult plants. A preliminary report on these experiments has already appeared (10).

[1] Contribution No. 827 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Associate Plant Pathologist.

EXPERIMENTAL METHODS

The field experiments were, for the most part, at Winnipeg, Manitoba. The soil at this station is classified as a heavy-textured black earth (4). It is a heavy clay, and although the surface soil is fairly uniform in texture the subsoil is quite variable. The studies on date and rate of seeding were made on summer-fallowed land, and those on depth of seeding were made on land that had been cropped continuously to wheat for a number of years.

The system of replication used in each experiment consisted of 4 or 6 randomized blocks, each date, rate, or depth of seeding occurring once in each block. Each plot consisted of 3 or more rows. The rows were spaced 1 foot apart. They were 18 feet long in the date and rate of seeding experiments, and 12 feet long in the depth of seeding experiments. Sufficient buffer rows were used in each plot to eliminate border effect. The seed was sown with a V-belt hand drill.

Data on plant emergence and seedling root rot were recorded about one month after the seed was sown. The methods, infection classes, and disease-rating formula used in obtaining these data were those employed by Greaney (8). Notes on the incidence of adult plant root rot were taken about 10 days before the plants ripened. These data were obtained as follows. The plants in one row of each plot were lifted from the soil and examined individually to determine the intensity of disease on each plant. In rating the plants for disease, 6 infection classes (0 to 5), indicative of the amount of basal lesioning and ranging from no lesioning to plants dead due to root rot infection, were used. The amount of disease in each plot was recorded as a root-rot rating. This was calculated from the formula $R = \frac{S}{N \times 5} \times 100$, where R = root-rot ratings, S = sum of individual plant ratings, N = number of plants examined, and 5 = highest individual plant rating. Dividing S by $N \times 5$ and multiplying by 100 gives a rating which represents the amount of disease in terms of the maximum possible for the plot. In other words, a root-rot rating of 0 indicates no disease and 100 a maximum amount of disease.

Yield data were obtained by harvesting and threshing separately one rod-row of each plot. The plant emergence, disease, and yield data of each experiment were treated statistically by the analysis of variance method, and the significance of the observed differences between dates, rates, or depths of seeding was determined.

EXPERIMENTAL RESULTS

Date of Seeding

Broadfoot (1), in a study of the effect of date of seeding on the incidence of foot rot of spring wheat caused by *Helminthosporium sativum* and *Fusarium spp.*, found that early-sown crops were more heavily infected with this disease than were late-sown crops. Robertson *et al.* (18) made a study of the relation of the date of seeding of winter wheat to dry-land foot rot and found that infection and severity of foot rot were greatest in the early

plantings. According to Russell (19) spring wheat, sown early, is more susceptible to the take-all (*Ophiobolus graminis* Sacc.) disease than if it is sown late. Kirby (13) states that late planting of winter wheat reduces the incidence of take-all.

An experiment designed to examine the influence of date of seeding on the development of Helminthosporium-Fusarium root rot was carried out at Winnipeg in 1936. Plots of Pentad wheat were sown at 4-day intervals from May 4 to June 12 (10 plantings). The experiment was repeated in 1937, 1938, and 1939. In these years the earliest planting was made on or near May 4. Thereafter, plots were sown at 4-day intervals until 10 plantings had been completed. The wheat variety Pentad was used in 1937 and 1938, and the variety Mindum in 1939. These varieties are susceptible to root rot. The seed used in each of the 4 experiments was heavily infected with *Helminthosporium sativum*. In 1936 and 1937 inoculum of *H. sativum*, prepared according to the methods described by Machacek and Greaney (15), was added to the rows at seed level as the seed was sown. No inoculum was added to the soil in 1938 and 1939.

Date of Seeding in Relation to Root-Rot Severity and Yield

The analyses of variance of the plant emergence, disease, and yield data of the date of seeding experiments are presented in table 1. A high degree of significance can be attached to the effects of date of seeding on plant emergence, disease development, and yield. For the disease data, the mean squares due to the interaction, dates × years, were much greater than the error mean squares, and, for these data, the mean squares for dates significantly exceeded the mean squares due to the interaction. In other words, the relative effect of date of seeding on the development of root rot was the same in the 4 years of the experiment. Table 1 shows that the effect of years on all properties studied was very great, as would be expected. To economize space, the analyses of variance data for individual years are not given. For each year, however, the results of the analyses showed highly significant differences between seeding dates for all the properties measured.

TABLE 1.—*Analyses of variance of plant emergence, root-rot, and yield data. (Date of seeding experiments, 1936 to 1939)*

Source of variance	Degrees of freedom	Mean squares			
		Percentage plant emergence	Seedling root-rot rating	Adult plant root-rot rating	Yield
Replicates	5	200.46	50.76	75.02	91.76
Years	3	8,815.37*	61,047.50*	7,534.06*	2,530.22*
Error (a)	15	39.78	42.97	37.75	51.63
Dates of seeding	9	3,466.05*	670.09*	2,188.07*	870.23*
Dates × years	27	201.98	145.66*	172.07*	81.25
Error (b)	180	168.82	34.36	41.74	75.12

* Exceeds mean square error, 1 per cent level.

In table 2 are given the average field-plot data for the different dates of seeding. It is apparent that the date at which the seed was sown in 1936, 1937, 1938, and 1939 exercised a marked influence on the development of root rot. Delay in seeding increased the incidence of the disease in seedling and adult wheat plants. The results obtained in each of the 4 years indicated that late stands of spring wheat were more severely affected by root rot than were early stands. In 1939, for instance, the average seedling root-rot rating for Mindum wheat sown on May 11, May 19, May 27, June 4, and June 12 was 14, 17, 23, 29, and 31, respectively, the corresponding figures for the adult plant root-rot rating being 16, 27, 29, 35, and 42, respectively.

TABLE 2.—*Influence of date of seeding on plant emergence, yield, and on the incidence of seedling and adult plant root rot in wheat. Mean results of experiments at Winnipeg in 1936, 1937, 1938, and 1939*

Date of seeding	Average mean daily soil temperature (° F.)[a]	Percentage seedling emergence	Seedling root-rot rating	Adult plant root-rot rating	Yield (bu. per acre)
May 7	43.5	67.8	45.5	30.8	35.7
May 11	45.0	64.8	46.8	30.8	33.2
May 15	49.2	65.8	49.0	41.0	27.4
May 19	47.8	69.8	48.5	40.0	25.6
May 23	50.0	69.2	52.5	48.2	26.2
May 27	50.0	67.2	54.2	46.0	25.3
May 31	50.3	66.2	56.8	51.5	25.1
June 4	50.1	61.5	58.5	54.5	22.3
June 8	50.4	60.5	57.2	52.2	19.9
June 12	50.7	58.8	60.8	56.0	16.8
Necessary difference, 5 per cent level	.....	7.9	3.6	4.0	5.3

[a] Soil temperature at depth of 4 inches.

It is clear from table 2 that time of planting had a very marked and significant effect on yield. There was a progressive decrease in yield from the earliest to the latest date of seeding. The fact that yield was greatest in the early seedings, and that it is inversely related to disease severity, indicates that root rot was a factor of considerable importance in reducing yields in the late-sown plots.

The data presented in table 2 indicate clearly that time of seeding did not influence appreciably the number of plants that emerged from the soil, except in the case of plantings made after May 31. Very late planting (June 12) significantly reduced percentage plant emergence.

Soil Temperature in Relation to Root-Rot Severity and Yield

The extensive investigations of Dickson (3), McKinney (16), and Johnston and Greaney (12) have indicated that wheat plants are blighted most severely by root-rotting fungi at relatively high temperatures. This finding is in agreement with that of several other investigators. Garrett (6), in a review of the extensive studies on the relation of soil conditions to root-

infecting fungi, points out that soil temperature affects a soil-borne disease such as common root rot of wheat not only directly through its effect upon host resistance, and upon the causal fungi, but also indirectly by influencing the factor of microbiological antagonism to the parasites. From the foregoing statements it is evident that soil temperature has a profound influence on root-rot development. As root rot was found in the present experiments to be much more severe in late sown crops than in crops from early seedings, soil temperature data were studied to ascertain to what extent this variability in root-rot infection may be related to soil temperature conditions at different seeding dates.

The average soil temperature data for the experiments of 1936, 1937, 1938, and 1939 are presented in table 2. A comparison of the mean daily temperatures for the different dates of seeding shows that the lowest soil temperature occurred at the earliest seeding date (May 7), and that there was a gradual rise in temperature as the season progressed. There was, however, a greater rise in soil temperature at the second and third seeding dates (May 11 and May 15) than at later dates. The differences in mean daily soil temperature between individual dates of seeding after May 15 are so small that they probably have no significance.

The evidence presented earlier showed that date of seeding had a very marked and significant influence on the development of root rot, and on yield, and, from what has just been said, it is evident that a close relationship exists between soil temperature and date of seeding. It would, therefore, be expected that a close association would exist between soil temperature and the incidence of root rot, and between temperature and yield. That this is so is clearly brought out by a comparison of the data in table 2.

With a view of determining the relation of soil temperature to root-rot development and plant growth, a statistical study was made of the temperature, disease, and yield data of the experiments. In this study the original field data of the 4 experiments were used. When mean daily soil temperatures and seedling root-rot ratings for each planting date were correlated, a significant positive correlation coefficient of $+0.401$ was obtained, indicating an increase in seedling blight with an increase in soil temperature. A significant positive coefficient of $+0.692$ was also found between the degree of adult plant root-rot infection and temperature. These results indicate clearly that soil temperature was closely associated with the development of root rot. The higher the temperature at time of seeding the higher was the degree of infection.

A highly significant inverse relation ($r = -0.622$) was found between soil temperature and yield. In other words, low temperature (early seeding) was associated with high yield. When the yield and the disease infection data were correlated, definitely significant negative coefficients were obtained, indicating that high yield was associated with low disease incidence. The foregoing statistical results, together with the average data in table 2, indicate clearly that soil temperature affects yield, and that part of the reduction

in yield in the late plantings was due to the influence soil temperature had on the development of root rot.

In the final analysis of the field data the seedling and adult plant root-rot ratings were correlated. A significant positive correlation coefficient of +0.562 was obtained. This result indicates that the susceptibility of the wheat varieties Pentad and Mindum to Helminthosporium-Fusarium root rot does not depend on their stage of development. They were equally susceptible in the seedling stage and at the stage of growth just prior to ripening (Table 2). It can be concluded, therefore, that the seedling reaction is a reliable index of the reaction of adult wheat plants to root rot caused by *Helminthosporium sativum* and *Fusarium spp.*

Rate of Seeding

Guerrapain and Demolin (11), Föex (5), Peyronel (17) and others in Europe, and Kirby (13) in America, report that the development of the take-all disease of wheat is favored by dense sowing. Broadfoot (1), in studies on the effect of crop rotation and cultural practice on the development of foot rot of wheat, was unable to find any close relation between rate of seeding and the incidence of the disease. However, the belief is commonly held in Manitoba that there is a close association between rate of seeding and the incidence of common root rot in crops of spring wheat. In order to obtain definite information on this point field experiments were carried out in 1938, 1939, and 1940.

TABLE 3.—*Analyses of variance of root-rot and yield data.* (*Rate of seeding experiments, 1938, 1939, and 1940*)

Source of variance	Degrees of freedom	Mean squares		
		Percentage plants diseased	Root-rot rating	Yield
Replicates	5	150.41	58.16	153.18
Years	2	415.18*	387.60*	547.26*
Rates of seeding	5	1,292.27*	593.02*	4,053.00*
Rates × years	10	64.64	27.54	20.33
Error	85	92.46	30.43	34.58

* Exceeds mean square error, 1 per cent level.

In each of these years seed of healthy Regent wheat was sown at 6 different rates, namely, 50, 100, 200, 300, 400, and 500 seeds to an 18-foot row. Three rows of each rate of seeding constituted a plot. Just before harvest, adult plant disease data were obtained in the usual way from the lateral rows of each plot. The center row of each plot was harvested for yield data.

The analyses of variance of the disease and yield data are given in table 3. For disease and yield the mean squares due to rates of seeding were significant. This means that density of sowing definitely affected the incidence of root rot and yield. The insignificant interactions for disease and yield indi-

cate that the effect of seeding rate on disease severity and on yield was the same in the 3 years of the experiment. The mean squares due to differences between years were all highly significant.

The variations in percentage of plants diseased, intensity of root-rot infection, and yield, due to rate of seeding, are shown in table 4. It is evident from this table that the incidence of root rot increased with an increase in the seeding rate. This is in accordance with general field observations in Manitoba and indicates that thinly-spaced wheat plants are less likely to be severely attacked by root rot than are thickly-spaced plants. Thus, the results of the experiments support the view that any cultural practice which tends to increase the vigor of individual wheat plants can be counted upon to reduce the amount of root-rot infection.

Table 4 shows that, in spite of a consistent increase in disease incidence, yield increased as the rate of seeding was increased from 50 to 400 seeds per

TABLE 4.—*Influence of rate of seeding on the incidence of root rot and yield in wheat in 1938, 1939, and 1940*

Rate of seeding per 18-foot row	Percentage of plants diseased				Adult plant root-rot rating				Yield (bu. per acre)			
	1938	1939	1940	Mean (1938–40)	1938	1939	1940	Mean (1938–40)	1938	1939	1940	Mean (1938–40)
50	59	69	68	65.3	12	8	15	11.7	8.1	31.4	14.6	18.0
100	68	70	72	70.0	16	14	18	16.0	11.5	39.4	20.3	23.7
200	72	78	96	82.0	17	21	26	21.3	16.4	42.1	27.9	28.8
300	88	90	99	92.3	23	23	35	27.0	17.5	41.1	26.7	28.4
400	96	90	99	95.0	27	25	33	28.3	17.5	48.3	34.2	33.3
500	96	97	100	97.7	29	23	37	29.7	18.0	45.8	34.3	32.7
N.D.[a]	11.2	13.1	9.6	6.8	4.8	5.4	6.1	3.9	1.6	6.9	4.6	4.2

[a] Necessary difference, 5 per cent level.

row. Above the 400-seed rate, however, there was no further increase in yield, a fact that is in agreement with the results of Machacek and Greaney (15) who found that individual plants in dense stands of wheat were less vigorous and tillered less than those in thin stands. This difference in vigor and tillering of plants accounts for the difference in their yielding ability, and also for the fact that very dense stands of wheat in the Winnipeg plots did not yield more than less dense stands. It is generally recognized, however, that, in Manitoba, the effects of rate of seeding on the development of root rot and on yield in wheat are likely to vary directly with the available soil moisture at seeding time, and also with the amount of rainfall from seeding time to harvest.

Depth of Seeding

Russell (19), in field experiments with the take-all disease of wheat, found that infection was increased by deep seeding. According to Broadfoot (1) depth of seeding had no significant effect on the incidence of Helminthosporium-Fusarium foot rot. To gain information as to the part

that depth of seeding plays in the development of common root rot, field studies were undertaken in 1943 and 1944.

In 1943, healthy seed of Thatcher and Red Bobs wheat was sown at 4 different depths in plots of infested soil at Winnipeg. The seeding depths used were 1 in., 2 in., 3 in., and 4 in. Each plot consisted of 3 rows, 1 foot apart, and 12 feet long. Two hundred seeds were planted in each row. Notes on the incidence of disease were taken on the center row of each plot in the usual way about 10 days before the plants ripened.

Evidence was obtained in 1943 which indicated that there was a positive relation between depth of seeding and the severity of root rot. Deep planting definitely increased the amount of root rot, and the increase was progressive. Taking 100 as the figure representing very severe root-rot infection, the mean values for plots of Thatcher and Red Bobs wheat sown at depths of 1 in., 2 in., 3 in., and 4 in. in 1943 were, respectively, 15.3, 21.6, 23.1, and 24.0.

TABLE 5.—*Analysis of variance of root-rot data. (Depth of seeding experiment, 1944)*

Source of variance	Degrees of freedom	Mean square	F value	5 per cent point	1 per cent point
Replicates	3	196.67	10.49	3.07	4.87
Varieties	3	219.58	11.71	3.07	4.87
Stations	1	2,320.67	123.77	4.32	8.02
Varieties × stations	3	97.33	5.19	3.07	4.87
Error (a)	21	18.75	.....	.....	.....
Depths of seeding	2	410.60	51.84	3.19	5.08
Depths × varieties	6	21.89	2.76	2.30	3.20
Depths × stations	2	5.48	.....	.....	.....
Depths × varieties × stations	6	11.67	1.47	2.30	3.20
Error (b)	48	7.92	.....	.....	.....

In view of these results it was decided to repeat the experiment in 1944 using 4 varieties (Thatcher, Regent, Renown, and Red Bobs), and 3 seeding depths (1 in., 2 in., and 3 in.). This enlarged field experiment was conducted at Winnipeg and Morden, Manitoba.

The analysis of variance of the root-rot data of the 1944 experiment is given in table 5. From this it is evident that the mean square for depths of seeding is highly significant. On the other hand, the mean squares due to the interactions, depths × stations and depths × varieties, failed to reach significance at the 1 per cent point. In other words, the effect of the depths of seeding was the same at both stations, and for the 4 varieties. The mean squares due to varieties and to stations were significant. This means that a high degree of significance can be attached to the differences observed between varieties and stations.

The mean root-rot ratings for each variety and station, together with the mean values over all stations and varieties, are given in table 6. It is clear that depth of seeding had a very marked and significant effect on the devel-

opment of root rot. At Winnipeg and Morden the incidence of the disease increased with depth of seeding. Comparisons between individual depths of seeding for all varieties and stations show that the differences observed in disease incidence between the different seeding depths were statistically significant.

It is evident from table 6 that, in so far as the development of root rot was concerned, depth of seeding was more important in some varieties than in others. That is, the varieties did not react to depth of seeding to the same degree. For instance, deep seeding definitely increased the amount of root

TABLE 6.—*Influence of depth of seeding on the incidence of root rot in certain varieties of wheat in 1944*

Variety, and depth of seeding in inches	Root-rot rating			
	Winnipeg	Morden	Average	Variety mean
Thatcher				
1	17.4	8.1	12.8	
2	24.0	11.0	17.5	16.6
3	26.2	12.8	19.5	
Renown				
1	23.2	11.4	17.3	
2	26.8	15.7	21.2	21.2
3	31.0	20.4	25.2	
Regent				
1	25.6	11.1	18.4	
2	27.2	16.0	21.6	22.9
3	34.2	23.1	28.6	
Red Bobs				
1	23.4	19.0	21.2	
2	23.5	23.2	23.4	23.1
3	27.0	21.3	24.6	
Necessary difference, 5 per cent level	4.7	7.2	5.0	6.0
All varieties				
1	22.6	12.4	17.5	
2	25.4	16.5	20.9	
3	29.9	19.4	24.6	
Necessary difference, 5 per cent level	2.4	3.6	2.5	

rot in the varieties Thatcher, Regent, and Renown, but only slightly affected root-rot development in the variety Red Bobs. The differences observed in root-rot rating between shallow (1 in.) and deep (3 in.) seeding of Red Bobs wheat were small and statistically insignificant. It is evident, therefore, that the relation between depth of seeding and the incidence of root rot may be modified considerably by the variety grown.

The varieties of wheat used in the experiment of 1944 ranked in order of resistance to root rot as follows: Thatcher, Renown, Regent, and Red Bobs. This ranking is in agreement with that obtained in earlier studies on the relative resistance of wheat varieties to common root rot (9). Thatcher was much more resistant to root rot than was any of the other varieties tested.

DISCUSSION

There is abundant evidence to indicate that in Manitoba the prevalence of common root rot of wheat varies considerably from season to season, and, in any one season, from district to district, and even from field to field. Local variation in the incidence of the disease suggests the possibility of control through crop and soil management. From the results presented in this paper it is evident that variation in the time, rate, and depth of seeding also influences the development of root rot. Early, thin, and shallow seeding reduced the intensity of the disease. This method of control appeared to be effective, and, in the case of common root rot of wheat, offers distinct practical possibilities.

There can be no doubt that there is a close interrelation between soil conditions and crop conditions, and between these and the amount of root rot that develops. The tendency for root rot to be more prevalent in wheat crops that are sown late, too deeply, or too thickly, arises as a result of the interaction of a number of factors. Soil temperature, moisture content, aeration, reaction, and soil nutrients are factors that play a part in modifying the relation between date, depth, and rate of seeding and the development of root rot. Furthermore, these factors are capable of affecting root-rot development not only directly through their effects upon the causal fungi, and upon host resistance, but also indirectly through their effects upon the complex microbiological flora of the soil. The important part different soil conditions play in the development and control of root diseases of wheat and other field crops has been adequately discussed by Garrett (7).

The opinion is widely held in Manitoba that dry seasons are usually seasons of heavy root-rot infection, and this opinion is supported by numerous observations made over a long period of time. For instance, low soil moisture conditions at time of seeding favor infection by retarding the germination of the seed, and the early growth of the wheat seedlings. Again, very low soil moisture content during the post-seedling stages of growth may injure the roots of the plants to such an extent that the entry of parasitic fungi is appreciably facilitated. On the other hand, high soil moisture at time of seeding followed by low soil moisture in the post-seedling stages of plant growth, favors root-rot development. As has been widely recognized, weakened plant growth caused by low moisture conditions constitutes a very important factor in the progress of the disease. The present field studies have indicated, however, that, in Manitoba, early seeding of wheat when the moisture content of the soil is relatively high promotes rapid and vigorous plant growth, and is an effective means of reducing the losses from seedling blight and root rot caused by *Helminthosporium sativum* and *Fusarium spp.* They have also emphasized the need of further field studies to elucidate the many factors responsible for variation in the incidence of root rot in relation to date, depth, and rate of seeding under local conditions.

SUMMARY

The results of field experiments, conducted in Manitoba in 1936, 1937, 1938, and 1939, on the relation between date of seeding of spring wheat and the incidence of root rot (*Helminthosporium sativum* and *Fusarium spp.*) indicated that early seeding reduced the incidence of the disease and increased yield. Soil temperature, as related to date of seeding, was positively associated with root-rot infection and negatively related to yield. Thus, low soil temperature (early seeding) was associated with low disease incidence and with high yield. The effect of high soil temperature on the incidence of root rot, with the resulting effect of the disease on the plants, was an important factor in reducing yields in late-sown crops of spring wheat.

The effect of rate of seeding on the incidence of root rot was studied in 1938, 1939, and 1940. A close relationship was found between rate of seeding and the development of root rot. Each year the severity of the disease increased with thickness of planting.

A close association was found between depth of seeding and the development of root rot. In experiments carried out in 1943 and 1944 root-rot severity increased with depth of planting. As a factor in the control of root rot, depth of seeding was more important in some varieties than in others.

Field experiments have indicated that agricultural practices which favor the rapid, vigorous growth of a wheat crop tend to reduce the intensity of root-rot infection. They have also demonstrated the practicability of reducing losses from Helminthosporium-Fusarium root rot in Manitoba by seeding spring wheat not too thickly or deeply, and at the earliest feasible date.

DOMINION LABORATORY OF PLANT PATHOLOGY,
WINNIPEG, MANITOBA, CANADA.

LITERATURE CITED

1. BROADFOOT, W. C. Studies on foot rot and root rot of wheat. III. Effect of crop rotation and cultural practice on the development of foot rot of wheat. Can. Jour. Res. **10**: 95–114. 1934.
2. CRAIGIE, J. H. Economic diseases of field crops in Manitoba. Economic Survey Board, Province of Manitoba, Project No. 16. 1939.
3. DICKSON, J. G. Influence of soil temperature and moisture on the development of seedling blight of wheat and corn caused by *Gibberella saubinettii*. Jour. Agr. Res. [U.S.] **23**: 837–870. 1923.
4. ELLIS, J. H. Soil types occurring in the Red River Valley plain. Scient. Agr. **15**: 276–286. 1935.
5. FÖEX, E. Sur le piétin du blé. Acad. Agr. France Compt. Rend. **5**: 543–548. 1919.
6. GARRETT, S. D. Soil conditions and the root-infecting fungi. Biol. Review **13**: 159–185. 1938.
7. ———. Root disease fungi. 177 pp. Chronica Botanica Co., Waltham, Mass., U.S.A. 1944.
8. GREANEY, F. J. The effect of phosphate deficiencies on infection of wheat by *Fusarium culmorum*. Can. Jour. Res. C, **16**: 27–37. 1938.
9. ———, J. E. MACHACEK, and C. L. JOHNSTON. Varietal resistance of wheat and oats to root rot caused by *Fusarium culmorum* and *Helminthosporium sativum*. Scient. Agr. **18**: 500–523. 1938.
10. ———. Influence of rate, depth, and time of planting spring wheat on the incidence of root rot. (Abstr.) Phytopath. **34**: 1001. 1944.

11. GUERRAPAIN, A., and A. DEMOLIN. Enquête et observations sur la maladie du piétin (pied noir des céréales). Jour. agr. prat. (n.s.) **26**: 566–567, 627–630. 1913.
12. JOHNSTON, C. L., and F. J. GREANEY. Studies on the pathogenicity of *Fusarium* species associated with root rot of wheat. Phytopath. **32**: 670–684. 1942.
13. KIRBY, R. S. The take-all disease of cereals and grasses caused by *Ophiobolus cariceti* (Berkeley and Broome) Saccardo. Cornell Agr. Exp. Sta. Memoir 88. 1925.
14. MACHACEK, J. E. An estimate of loss in Manitoba from common root rot in wheat. Scient. Agr. **24**: 70–77. 1943.
15. ————, and F. J. GREANEY. Further experiments on the control by seed disinfection of root-rotting fungi in wheat. Phytopath. **31**: 379–394. 1941.
16. MCKINNEY, H. H. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Jour. Agr. Res. [U.S.] **26**: 195–218. 1923.
17. PEYRONEL, B. Il "mal del piede" dei cereali. Boll. R. Staz. Path. Veg. **6**: 285–336. 1926.
18. ROBERTSON, D. W., O. H. COLEMAN, J. F. BRANDON, H. FELLOWS, and J. J. CURTIS. Rate and date of seeding Kanred winter wheat and the relation of seeding date to dry-land foot rot at Akron, Colo. Jour. Agr. Res. [U.S.] **64**: 339–356. 1942.
19. RUSSELL, R. C. Study of take-all (*Ophiobolus graminis*) of wheat. Rept. of Dominion Botanist, Canada Dept. Agr., Ottawa. **1930**: 78–81. 1931.

EXPLORATORY EXPERIMENTS WITH THE BIG-VEIN DISEASE OF LETTUCE

DEAN E. PRYOR[1]

(Accepted for publication October 25, 1945)

In 1934, Jagger and Chandler (1) predicted that big vein might become troublesome in lettuce grown on the heavier soils of California and Arizona. At that time the disease was known to be present only in those western states. However, big vein has now been reported from several states along the Atlantic Seaboard (2, 4). It is not known whether this malady was recently introduced into this region, or whether it was merely differentiated from other lettuce diseases after Jagger and Chandler published their report. Since 1934, big vein has not increased particularly in the Imperial Valley, California, or in the Arizona section, but has become more prevalent in the Salinas-Watsonville district. The increased importance of the disease made it seem desirable to investigate further its nature and behavior. Consequently in 1939 several lines of investigation on the disease were initiated with emphasis upon those factors that influence its behavior in trials for disease resistance. Because of the war most of the work on big vein was suspended in 1942, but it seems desirable to publish the results obtained thus far. Although the data in many instances admittedly are only suggestive, they may be of some aid to workers who study the disease.

TRANSMISSION EXPERIMENTS

Insect Transmission

In February, 1941, several flats of lettuce plants from breeding lines were grown from seed in a greenhouse being used for big-vein experiments. The flats were placed on a bench filled with sterilized soil. These plants were transplanted to a disease-free field. Big vein appeared shortly after transplanting and, on June 2, 12.6 per cent of about 2,000 plants were diseased. Since it seemed that insect transmission in the greenhouse may have been the means of infection, attempts were made to transmit the disease with the aphids *Macrosiphum solanifolii* Ashm., *Myzus convolvuli* Kalt., and *M. persicae* Sulz. These species are present on lettuce from time to time in greenhouses at La Jolla. In 18 experiments, with various methods of transfer and different feeding periods, big-vein symptoms occurred in only a very few plants. Consistent transmission even to a low percentage of plants could not be obtained.

Thompson, Doolittle, and Smith (5) obtained very similar results with certain leaf-feeding aphids. On the other hand, their preliminary experi-

[1] Associate Plant Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture. The author wishes to acknowledge the assistance of W. W. McCoy, formerly Field Aide, Division of Fruit and Vegetable Crops and Diseases, in parts of this work.

ments with the root aphid *Pemphigus lactucae* (Fitch) indicate that this insect may be an efficient vector. Since root aphids have not been found in the greenhouses at La Jolla, it would seem that other vectors of big vein might exist.

Mechanical Transmission

In 10 trials, involving about 700 plants including checks, no consistent transmission was obtained with leaf juice by (*1*) leaf-rubbing methods using materials such as cotton, cheesecloth, and carborundum; (*2*) leaf-punctures using needles through drops of juice from diseased plants and hypodermic needles thrust into the larger veins and into the stem; or by (*3*) root inoculation by hypodermic needles, or soaking roots in leaf juice from big-vein-infected lettuce.

Seed Transmission

Plants grown on disease-free soil from seed soaked for 24 hours in juice from big-vein-infected plants did not contract the disease.

In one lot of seed from infected lettuce, 18 of approximately 1,000 plants developed big vein. Other extensive trials, however, gave no indication of seed transmission. In view of these results, it appears that big vein is rarely if ever, seed-transmitted.

Soil Transmission

By Soil Extracts. To 3½ kg. of highly infective soil was added 8 liters of tap water. The mixture was thoroughly stirred and then allowed to settle. The apparently clear supernatant liquid was siphoned off, care being taken to collect no macroscopically visible soil particles. This liquid was divided into two parts, one of which was filtered through a medium-grade Mandler filter. The filtered and unfiltered solutions were each divided into five equal parts and added respectively to two groups of five pots of disease-free soil in which small lettuce plants were growing. As a control a similar lot of soil was autoclaved for 2 hours at 15 pounds' pressure, then used like the infective soil to prepare unfiltered and filtered solutions for pot application. In each case the leached soil was also planted to lettuce.

After about 2 months many big-vein plants occurred on the infective, nonautoclaved, leached soil and none on the autoclaved, leached soil. Both of the leachates from autoclaved soil and the filtered leachate from infective soil produced no disease when added to big-vein-free soil (two experiments). However, the unfiltered leachate from infective soil produced four big-vein plants in a total of 83 plants in noninfective soil.

The results corroborate those of Jagger and Chandler (1) in that leaching does not materially decrease soil infectivity. The leaching experiments were too few to warrant definite conclusions, but they seem to indicate either that some infective soil was carried in the unfiltered extract or that the infective particles are nonfiltrable, or that they are absorbed by bodies in the soil that are macroscopically invisible but large enough to be removed by a medium Mandler filter.

By Contamination of Soil with Lettuce Leaves Having Big-Vein Symptoms. In the various experiments, big-vein plants appeared occasionally on soil supposedly not infective. They were cut off at the soil level at the conclusion of the experiment and the pot replanted with lettuce started in disease-free soil. Almost invariably replants developed big vein. To check further on the possibility that diseased plant remains might contaminate noninfective soil, two experiments were run. In the first trial, 650 gm. of coarsely chopped big-vein leaves were mixed with enough soil to fill 15 6-inch pots; in the second, 1,000 gm. were used for 10 pots. The same number of control pots received equal treatments except that healthy lettuce leaves were used. Four plants from disease-free soil were then transplanted to each pot.

All of the check plants remained healthy in both experiments. After about 2½ months, five out of 48 plants surviving in soil mixed with diseased leaves in the first experiment showed big vein; in the second experiment five out of 40 plants had the disease. These results suggest that the infective principle causing big vein can be transmitted to soil by diseased leaves.

EXPERIMENTS ON THE BEHAVIOR OF THE INFECTIVE PRINCIPLE IN THE SOIL

Direct Seeding versus Transplanting in Big-Vein-Infective Soil

In trials for resistance to big vein and in other experiments on infective soil, it is necessary to obtain as high a degree of infection as possible. For this reason, it was desired to learn whether direct seeding in or transplanting to infective soil produced the greatest number of diseased plants. Three trials involving a total of 112 plants from seed and the same number from transplants indicated a very slight tendency for more big vein to develop in the transplants. Since transplants are generally used in the big-vein susceptibility tests at this station, this line of investigation was dropped.

Spread Through the Soil from Diseased Plants

To discover whether big vein would spread from a diseased plant to adjacent healthy plants, one small big-vein plant and three healthy plants were set near the rim and 90° apart in each of eight 6-inch pots, in each of three experiments. The plants were grown for 2 to 2½ months. All of the big-vein plants showed the disease throughout the length of each run. In the first trial, two of the 24 healthy plants developed big vein; in the second, one doubtfully diseased plant occurred; and in the third, the originally healthy plants remained so during the course of the experiment.

It is possible that the disease development in these experiments might have resulted from insect transmission. In any event, it appears that big vein spreads very slowly, if at all, through undisturbed soil.

Effect of Drainage on the Development of Big Vein

In earlier studies on soil moisture in relation to big vein (3) it was noted that in soil from the Imperial Valley, California, salt concentrations built

up to such a point that plant growth was reduced. In some experiments not reported in the earlier paper there was a rather pronounced decrease in infectivity as the salt concentration increased. An analysis[2] was made of two lots of soil identical except that salt had been built up in one through continued use in pots without drainage and that this high-salt soil was considerably less infective. The total salt concentration in the highly infective soil was less than $\frac{1}{3}$ that in the soil of low infectivity (Table 1). The very great difference in amount of sulphate in the two lots of soil is notable.

Since the increase in salt content and decrease in infectivity apparently resulted from use of pots without drainage, four experiments were run in which the development of big-vein plants in drained and nondrained pots was recorded. In general there was a slight tendency for fewer diseased plants to appear in the nondrained pots than in the drained but in only one trial was this difference statistically significant and the mean difference of

TABLE 1.—*Analysis of two lots of soil differing in big-vein infectivity and in salt concentration*

Soil infectivity	Parts per million in soil extract								
	N[a]	P[b]	K[c]	Ca	Mg	Fe	SO_4	Cl	Total
Low	106	15	62	200	Tr.	15	500	50	948
High	54	4	60	150	Tr.	13	Tr.	10	291

[a] Includes NO_3, NH_4, NO_2.
[b] Includes reserve P.
[c] Includes reserve K.

all tests was nonsignificant. Only one crop of lettuce was grown in each pot. It may be that had two or more crops been grown in each pot the differences would have become more pronounced.

Time Required for Infection in Big-Vein-Infective Soil

Lettuce seed was sown in highly infective big-vein soil. Two weeks after planting the lettuce was up but only the cotyledons had unfolded. Twenty plants were removed at this time, washed thoroughly in tap water and transplanted to disease-free soil in 6-inch pots with four plants per pot. At weekly intervals thereafter, an additional 20 plants were removed, washed, and transplanted. When big vein appeared in lettuce on the big-vein soil, transplanting was discontinued.

The amount of big vein occurring in the transplants about 10 to 16 weeks after seeding is shown in table 2. This type of experiment is open to the criticism that it is impossible to be sure that all of the infective soil was removed in washing. Furthermore, since nothing is known about where infection takes place in the root system or how fast the causal entity moves after infection there is the possibility that the infected portion of the root systems may have been separated from healthy portions in the transplanting

[2] The author is indebted to John H. Pryor, Growers Ice and Development Company, Salinas, California, for making this analysis.

process. Nevertheless, it is apparent that some plants did not become diseased within 6 weeks from seeding while others seem to have been infected almost as soon as they emerged from the seed. Earlier observations (3) indicated that big vein develops best in vigorously growing plants. Since transplanting undoubtedly slows down the growth rate for a time it might be expected that when those plants with the longer time intervals in infective soil were held in disease-free soil for equivalent lengths of time, a greater proportion of big vein would develop in the later transplants. The last two columns of table 2 show this to be true. From this work it may be tentatively concluded that at least four weeks is required in big-vein soil for a large proportion of the plants to become infected.

Effect of Soil Dilution on Infectivity

In the first of these experiments, dilutions of infective big-vein soil with autoclaved big-vein soil did not reduce disease at the maximum dilution of

TABLE 2.—*The amount of big vein appearing in lettuce after transplanting seedlings grown from seed sown in infective soil*[a]

No. weeks in infective soil	Counts after variable time in disease-free soil				Number of big-vein plants after 10 weeks in disease-free soil	
	No. weeks in disease-free soil	Number of big-vein plants				
		Test 1	Test 2	Test 3	Test 1	Test 2
2	8	2	4	2	2	4
3	7	3	4	11	3	5
4	6	6	11	11	6	15
5	5	6	9	15	8	14
6	4	3	14	....	6	18

[a] Twenty plants grown at each exposure in each test.

1 part infective soil to 32 parts of sterilized soil. In another experiment the maximum dilution of 1 part to 800 reduced disease only slightly. The autoclaved soil in each case had only healthy plants. It is unwise to generalize from such few data, but this high dilution with little reduction in disease may indicate that the soil on farm implements might be effective in spreading the disease.

Persistence of Big-Vein Infectiveness in Soil

In 1936 a lot of big-vein soil was collected in the Imperial Valley of California and stored in a large, covered, galvanized-iron can in a dry place. From 1940 to 1944 small portions of this soil were removed periodically, tested for infectivity, and then discarded. Parallel checks were not run; however, checks in other experiments seldom became diseased and when they did the amount was never greater than 1 or 2 per cent. It is evident that big vein will persist with no decrease in infectivity for at least eight years in stored, air-dry soil (Table 3).

TABLE 3.—*Persistence of big-vein infectiveness in stored, air-dry soil*

No. years in storage	4	5	7	8
No. plants tested	33	24	6	24
Percentage of plants with big vein	33	54	83	75

The Influence of Soil Temperatures on the Production of Big-Vein Symptoms

Methods. Five "Wisconsin-type" soil-temperature tanks each containing eight $6\frac{1}{2} \times 9$-inch round galvanized-iron culture cans were used for the temperature studies. The tanks were located outdoors and a movable shelter was employed to cover them when there was probability of rain. Temperature was controlled by hot or cold water introduced through a perforated pipe running lengthwise and parallel to the bottom of the tank and about $1\frac{1}{2}$ inches below the bottoms of the cans. When the tanks were operated below air temperatures, the water was cooled by a small refrigeration unit. The addition of hot or cold water was controlled by solenoid valves actuated by poultry-incubator-type thermostats[3] which were sensitive to about $\pm 0.5°$ C. Temperatures used were 14°, 18°, 22°, 26°, and 30° C.

The infective soils were the ones employed in the moisture studies (3). The soil line in the cans was slightly below the water line in the temperature

TABLE 4.—*Effect of soil temperature on the development of big-vein symptoms in lettuce*

Experiment No.[a]	Time of the experiment	Percentage of big-vein plants at various soil temperatures (32 plants initially at each temperature in each experiment)					Total percentage of big-vein plants	Average daily air temperature[b]		
		14° C.	18° C.	22° C.	26° C.	30° C.		Maximum °C.	Minimum °C.	Mean °C.
1	Dec. 31, 1941, to Feb. 18, 1942	7	22	32	34	17	23	.....	.....	.....
2	Mar. 5 to May 1, 1942	38	48	60	48	10	41	18.1	8.5	13.3
3	Nov. 6, 1942, to Jan. 11, 1943	88	90	93	68	38	78	18.9	7.6	13.3
4	Feb. 26 to May 15, 1943	87	93	96	22	5	64	18.2	10.5	14.3
5	July 20 to Sept. 14, 1943	88	97	75	16	0	56	23.6	16.2	19.9
6	Nov. 22, 1943, to Feb. 19, 1944	84	90	94	54	10	67	17.2	6.8	12.1
7	Apr. 5 to June 13, 1944	94	84	97	34	21	67	18.6	11.3	14.9

[a] Experiments one through five were with Salinas soil; six and seven were with Imperial Valley soil.

[b] The mean temperature is the average of the daily extremes. No reliable air temperature records were available for experiment number one. The refrigeration unit was out of order from May 2, 1944, to June 13, 1944, when experiment 7 was run.

[3] Type SA9–3Z obtained from Lyon Rural Electric Company, San Diego, California.

tanks and 1¼ inches below the rims of the cans. Four lettuce plants with one or two true leaves were transplanted to each can after the soil had reached the desired temperature. The top of the soil was insulated with 30 gm. of rock wool. Enough water was added at short intervals through a central perforated pipe in each can to maintain the moisture at 75 per cent of the moisture-holding capacity of the soil (3). Plants were examined for big-vein symptoms at each watering. When there was evidence of crowding or head formation the experiment was discontinued.

Results. The data from several experiments are in table 4 together with the air temperatures prevailing during the trials. It is apparent that, with the exception of experiments number one and five, the greatest percentage of disease developed was at 22° C. There was generally a sharp decrease in the amount of big vein at 26° and 30° C. and some decrease at 14° and 18° C.

From reports in the literature (1, 4) air temperature also seems to have an influence on the production of big-vein symptoms and might have caused the discrepancy mentioned above. With this possibility in mind, air temperature records had been kept during most of the trials. No adequate records were available during the course of the first experiment, but from those at hand it appeared that the minimum air temperature at least was somewhat lower than in the other trials. Observations also indicated that the infectivity of the soil in the first two experiments was not so high as in the later ones. With these facts in mind, the apparent suppression of symptoms at higher soil temperatures in the fifth experiment might be explained as an effect of the substantially higher air temperature. Comparing the air temperatures with the amount of big vein at different soil temperatures, it is evident that concurrently with the higher air temperatures during July, August, and September, in experiment number 5, the optimum soil temperature was lowered to around 18° C. There is also a slight indication that the higher optimum soil temperature during the first experiment might have resulted from lower air temperature.

In each of the temperature experiments the initial number of lettuce transplants was 160, or 32 for each treatment. In some cases the survival was not very satisfactory, particularly at the higher soil temperatures (26° and 30° C.). This is not surprising since lettuce is known to be a low-temperature crop.

Discussion. Jagger and Chandler (1) and Thompson and Doolittle (4) observed that low temperature favors the development of big-vein symptoms. Since big vein is a soil-borne disease, it was thought that soil temperature might be more important than air temperature in producing this effect. While preliminary results reported here indicate that big vein will develop in lettuce plants grown at a constant soil temperature of 30° C., Thompson and Doolittle (4) observed that greenhouse air temperatures of 65° to 75° F. during the day and 50° to 60° F. at night prevented the appearance of big-vein symptoms in lettuce leaves. When the temperature was lowered to 50° to 60° F. during the day and to 45° to 50° F. at night they found that big

vein developed in some plants. Greenhouse temperatures again raised to the higher level suppressed symptoms. The fifth experiment reported in the present paper was carried out when air temperatures were comparable to those employed by Thompson and Doolittle (4) yet some big vein was evident at a soil temperature of 26° C. (78.8° F.), considerable was present at 22° C. (71.6° F.), and still more occurred at lower soil temperatures. During the course of this experiment, the range of maximum air temperature was 70° to 83° F. and the range of the minimum was 55° to 68° F. The discrepancy in results obtained in the two investigations possibly may have been due to the maintenance of a longer period of high air temperature in the greenhouse than occurred in the present work. Other explanations may be forthcoming with further research.

Indications are that when air temperature is favorable for the production of big-vein symptoms a soil temperature of about 22° C. is optimum for disease development in the leaves. However, considerably more work is necessary before the effect of air temperature and the combined effects of soil and air temperatures can be properly evaluated.

SUMMARY

Experiments using three species of leaf-feeding aphids, various methods of mechanical inoculation, lettuce seed soaked in leaf juice from diseased plants, and seed harvested from diseased plants resulted in no consistent transmission of big vein. The unfiltered leachate from infective soil when added to disease-free soil produced big vein in four of 83 plants grown thereon. The filtered leachate (medium Mandler filter) caused no disease. Big vein was not eliminated from infective soil by leaching. Symptoms appeared in plants grown on big-vein-free soil to which chopped diseased leaves were added. None appeared in comparable checks.

There was little difference in the amount of big vein developing in transplants and in lettuce seeded directly in infective soil. Trials in which one big-vein plant was grown for 2 to 2½ months adjacent to three healthy plants in a 6-inch pot filled with disease-free soil indicated that big vein spreads very slowly if at all through the undisturbed soil during the period used. A slight tendency existed for fewer diseased plants to appear in undrained pots filled with Imperial Valley soil than in drained pots but in only one experiment was this difference statistically significant. Transplanting lettuce at intervals from infective to noninfective soil indicated that some big-vein infection takes place within two weeks from seeding; under the conditions imposed, at least four weeks' growth in big-vein-infective soil was required for a large portion of the plants to become infected. Exploratory trials indicated that diluting 1 part of big-vein soil with 800 parts of autoclaved soil reduced disease incidence only slightly. Data obtained thus far show that stored, air-dry soil remains infective for at least eight years. When air temperature was favorable, the optimum soil temperature for development of big vein appeared to be about 22° C. (71.6° F.), with some

big vein occurring at all soil temperatures from 14° to 30° C. However, air temperature also seemed to have some effect on symptom expression.

U. S. HORTICULTURAL FIELD STATION,
LA JOLLA, CALIFORNIA.

LITERATURE CITED

1. JAGGER, IVAN C., and NORMAN CHANDLER. Big vein, a disease of lettuce. Phytopath. 24: 1253–1256. 1934.
2. NANCE, NELLIE W. Diseases of plants in the United States in 1939. U. S. Dept. Agr., Plant Disease Reporter Supplement 128: 210–378. 1940.
3. PRYOR, DEAN E. The big vein disease of lettuce in relation to soil moisture. Jour. Agr. Res. [U.S.] 68: 1–9. 1944.
4. THOMPSON, ROSS C., and S. P. DOOLITTLE. Influence of temperature on the expression of big vein symptoms in lettuce. Phytopath. 32: 542–544. 1942.
5. ———, ———, and FLOYD F. SMITH. Investigations on the transmission of big vein of lettuce. Phytopath. 34: 900–904. 1944.

PEACH BLOTCH[1]

R. S. WILLISON

(Accepted for publication November 1, 1945)

In the course of an intensive survey of peach orchards in a two-square-mile block near Winona, Ontario, conducted in the years 1937 to 1940 in connection with some studies of peach yellows, mosaic-like variegations were observed in 1940 on a single three- or four-year-old tree of an unknown large-flowered variety. The variegations were well-defined pale green to yellowish green areas very variable in size and shape, ranging from numerous angular spots scattered over the leaf surface (Fig. 1, A) to larger, irregular blotches usually fewer in number (Fig. 1, B, C). A few leaves were marked only by a yellowing along the larger veins (Fig. 1, H). Affected leaves as a rule were not distorted though some had ragged margins (Fig. 1, D), resulting from marginal scorch. The fruit was not affected and no marking or stunting of twigs was observed. Peach blotch has been adopted as the name for this disorder, as being characteristic of the predominating symptom.

Preliminary inoculations of peach seedlings by budding, in 1940, demonstrated the graft-transmissible nature of the variegation, symptoms appearing on the seedlings the following spring. In 1942, transmission tests were extended to include several peach, plum, and cherry varieties which, except for one seedling cherry, were inoculated by the double-budding technique (5). In these tests, peach, Myrobalan, and Mahaleb seedlings were used for stock.

Peach seedlings varied considerably in their response to blotch with respect both to symptom expression and to susceptibility. A few were resistant, showing only slight symptoms on early leaves, but the majority were more or less susceptible, symptoms being found on leaves of all ages. The markings were, in scope and variety, typical of those observed on the original tree, with a few additional variants. One of the latter was an almost symmetrical chlorotic blotch centered about and extending along the mid-rib and tending to feather out along the lateral veins (Fig. 1, F). Inversions of this pattern also occurred (Fig. 1, G) in which the chlorosis was peripheral. Occasionally leaves were completely chlorotic, except for a few, scattered, darker green islands (Fig. 1, E), an inversion of the angular spot pattern found on the original tree. Usually most patterns could be found on a single seedling though sometimes one type of pattern tended to predominate. The amount of scorch varied from seedling to seedling and from season to season. Though large necrotic spots were sometimes to be found, necrosis was typically marginal.

Of the peach varieties tested, Elberta and Rochester were most susceptible. The former displayed symptoms much like those on the original tree,

[1] Contribution No. 830 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

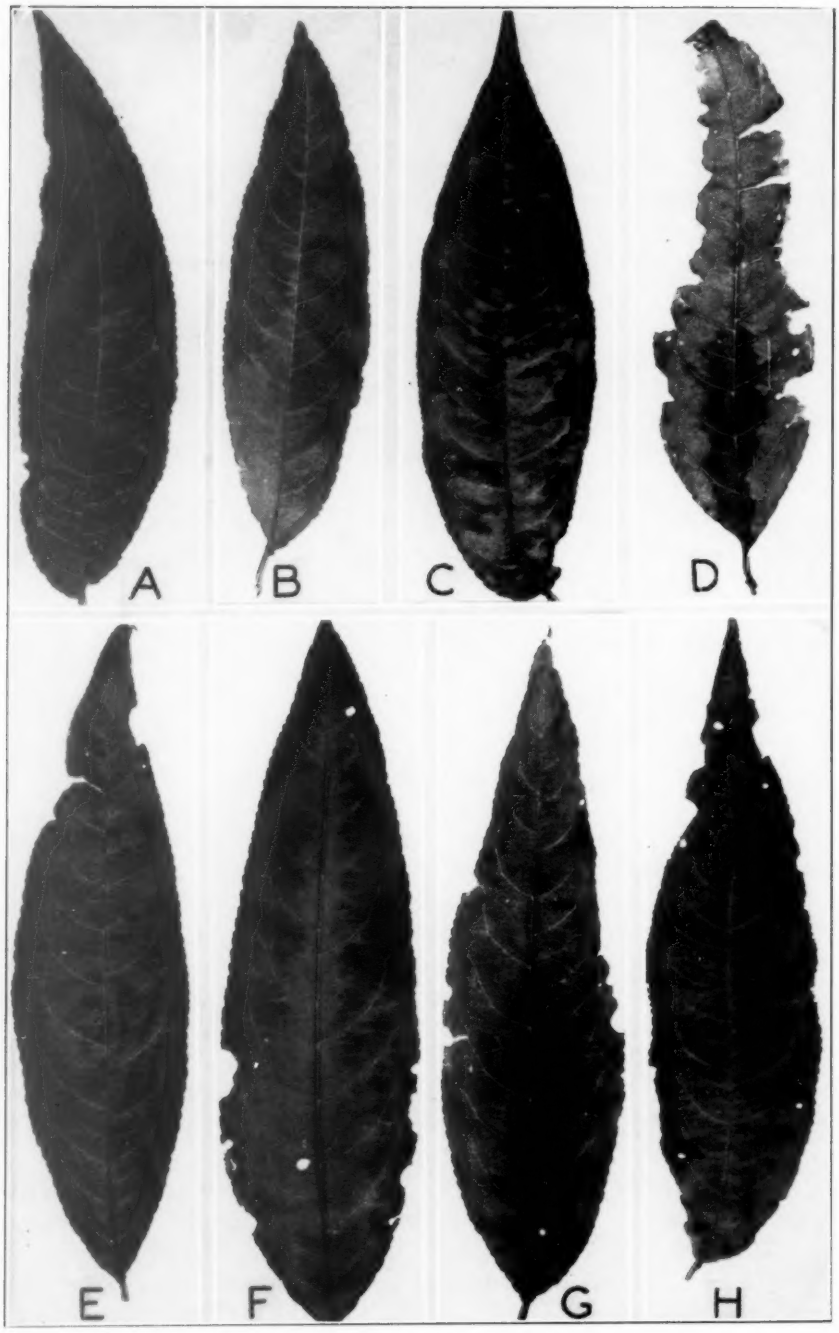


FIG. 1. Pattern variations of peach blotch on peach leaves. A. Small, angular, chlorotic spots. B, C. Irregular blotch patterns. D. Peripheral chlorosis and emargination by scorch. E. Inversion of A. F. Central feathery chlorosis. G. Peripheral chlorosis, inversion of F. H. Faint general chlorosis along veins. A, B, H, from original tree; C, D, E, F, G, from inoculated seedlings.

whereas in the latter, the feathery pattern (Fig. 1, F) predominated. Vedette showed slight blotching in some seasons and little or none in others. Golden Jubilee was almost symptomless, though a few early leaves sometimes had either a mild mosaic pattern or yellowing of veins. Few if any symptoms were observed on Peregrine, a white-fleshed variety imported from England. On all peaches, the patterns were either pale green, yellowish green, or greenish yellow, but never white, and no indications of symptoms were seen on flowers or twigs.

To date, transmission experiments with plum, apricot, and cherry varieties and the cherry seedling have given negative results in at least two seasons since inoculation except for a vague and doubtful blotching on Italian prune. In most cases, the inserted diseased peach buds grew, and even where they did not, union with the stock took place so that transmission of the virus to each of the test varieties was possible. The varieties used in these experiments included: *Prunus domestica* var. Italian prune, German prune, Reine Claude, and Lombard; *P. salicina* var. Abundance; *P. cerasifera,* Myrobalan seedlings; *P. armeniaca* var. Niagara; *P. avium* var. Black Tartarian, Napoleon, and seedling; *P. cerasus* var. Montmorency, and *P. mahaleb* seedlings. The double-budding tests, however, should be supplemented by inoculation of the same varieties after they have been indexed and established as nursery stock. Whether or not plums and cherries can act as carriers of blotch has not yet been determined.

In 1944, what appeared to be a second natural occurrence of blotch was found on sucker growth near the crotch of a tree of the Marigold variety in an orchard near Hamilton, Ontario. In this case, the predominating symptom type was the central, symmetrical, feathery blotch or some modification thereof. Inoculation of peach seedlings has demonstrated transmissibility, but the effects of this virus on other hosts are yet to be worked out.

Two other infectious variegations of the peach, mottle and calico, have been investigated by Blodgett (1, 2). Reeves (4) has also found isolated cases of a calico-like disorder on peach in Washington. Blodgett's descriptions of mottle and calico and his illustrations of calico indicate that these two diseases bear considerable resemblance to peach blotch, both in their foliage symptoms and in the rarity of their occurrence in nature. On the other hand, these three diseases and presumably their causal viruses are not identical. For example, the peach mottle virus (1) causes not only severe mottling, deformity and necrosis of leaves, and dwarfing of trees of sweet cherry but also die-back and gummosis of Montmorency, whereas the blotch virus and the calico virus (2) have had no apparent effect on cherry varieties. Calico also differs from blotch, though less markedly, in that its patterns become "brilliant-yellow, almost papery-white" and appear on twigs and fruits as well as on leaves. Furthermore, among the symptoms of calico no mention is made of the marginal lacerations and scorch associated with both blotch and mottle.

As these three peach disorders are not of great economic importance at

the present time, their chief claim to interest lies in the possible implications of the characteristics they have both individually and in common, considered in relation to their distribution. Their foliage symptoms on peach suggest that the diseases may be caused by virus strains, more or less closely related but differing in virulence. The differences between the diseases could then be construed as differences in degree rather than in kind. It is difficult, however, to see how such strains could arise when the disorders they produce occur as virtually isolated cases in widely separated districts. It is, of course, possible that the diseases may be sporadic infections by pre-existent viruses not ordinarily transmitted to peach. In such an event there would seem to be undue stretching of coincidence to expect sporadic infections of that type, even under necessarily highly exceptional conditions, to be confined to single trees in an orchard or district, particularly if more than one virus is involved. Blodgett's suggestions (2) that some viruses "may be originating within rather than being contracted by certain host plants" and that "the differences between perpetuated abnormalities and transmissible diseases, such as calico, may be, after all, fundamentally rather slight" deserve some consideration, especially in the light of recent work by Woods and Du Buy (3, 6). These investigations indicate the possibility that infectious as well as noninfectious variegations may arise through alterations in the chondriosomes or plastids. Such an explanation of the origin of the diseases under discussion could account both for their peculiar distribution as well as for their points of similarity and dissimilarity. However, until more than circumstantial evidence is forthcoming, the relationships between the causal viruses themselves and between the viruses and their hosts must remain enigmatic.

DOMINION LABORATORY OF PLANT PATHOLOGY,
ST. CATHARINES, ONTARIO, CANADA.

LITERATURE CITED

1. BLODGETT, E. C. Mottle, pp. 26–27 in Hildebrand E. M., G. H. Berkeley, and D. Cation. Handbook of virus diseases of stone fruits in North America. Mich. Agr. Exp. Stat. Misc. Publ. 1942.
2. ———. Peach calico. Phytopath. 34: 650–657. 1944.
3. DU BUY, H. G., and M. W. WOODS. Evidence for the evolution of phytopathogenic viruses from mitochondria and their derivatives. II. Chemical evidence. Phytopath. 33: 766–777. 1943.
4. REEVES, E. L. Virus diseases of fruit trees in Washington. Wash. State Dept. Agr. Bull. 1. 1943.
5. WILLISON, R. S. Strains of prune dwarf. Phytopath. 34: 1037–1049. 1944.
6. WOODS, M. W., and H. G. DU BUY. Evidence for the evolution of phytopathogenic viruses from mitochondria and their derivatives. I. Cytological and genetic evidence. Phytopath. 33: 637–655. 1943.

GROWTH AND OVERWINTERING OF XANTHOMONAS VESICATORIA IN ASSOCIATION WITH WHEAT ROOTS [1]

STEPHEN DIACHUN AND W. D. VALLEAU

(Accepted for publication November 20, 1945)

In previous reports it has been demonstrated that the tobacco leaf-spot bacteria, *Bacterium tabacum* and *B. angulatum*, can form colonies on roots of wheat and other unrelated plants, and, furthermore, that these two organisms can maintain themselves in field soil indefinitely, apparently in association with roots (6, 7).

The investigation has been extended to find out, if possible, whether plant pathogens other than the tobacco leaf-spot bacteria can perpetuate themselves in association with roots. The selection of species to be tested is somewhat limited by the method used in the study. To determine the presence or absence of pathogenic bacteria in soil or on roots, an aqueous suspension of the suspected soil or roots is poured on an artificially water-soaked leaf of the appropriate host. The leaf is water-soaked by squirting a stream of water from a hypodermic syringe against the lower surface; water-soaking occurs only if the stomata are open, and remains only a few minutes. Water-soaking, if properly done, does not seem to injure leaf cells, as indicated by the fact that when tobacco-mosaic virus is poured over water-soaked leaves of mosaic-resistant plants (N N) no necrotic spots result (2). If a suspension of bacteria is placed on such a temporarily water-soaked leaf some of the bacteria enter the leaf, in the water-soaked area, and, if pathogenic, they subsequently produce leaf spots (1); but temporary water-soaking does not increase susceptibility to nonpathogenic organisms. Thus, development of spots on leaves inoculated in this way with suspected roots or soil shows that pathogenic bacteria were present in the tested material, and failure of spotting indicates absence of the bacteria, or perhaps presence in very small numbers.

Xanthomonas vesicatoria, *X. phaseoli* var. *sojense*, and *Bacterium medicaginis* var. *phaseolicola* were selected for study. They cause common, widely distributed diseases, and appear to differ somewhat among themselves in the method of overwintering. Epidemics of halo blight of beans, caused by *B. medicaginis* var. *phaseolicola* are generally believed to be initiated largely by seed-borne organisms (4). Also, much of the primary infection caused by *X. vesicatoria*, is thought to be traceable to contaminated seed, but because epidemics have occurred in plants grown from seed believed to be clean, there is reason to believe that primary infection can originate in some other unknown source, perhaps contaminated soil (3). Primary inoculum for outbreaks of soybean bacterial pustule, caused by *X. phaseoli*

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

var. *sojense*, is commonly thought to come from overwintered organisms in fallen diseased leaves (5).

In the present report are given the results of tests designed to determine whether these 3 organisms can grow on roots and also overwinter in soil in association with wheat roots.

Colony Formation on Roots. The ability of these species of bacteria to grow on roots was tested on 4 species of plants. Seeds of Thorne wheat, Rutgers tomato, Stringless green-pod bean, and Macoupin soybean were surface-sterilized by soaking 2 hours in a 1.25 per cent solution of NaOCl, then germinated on sterile moist paper towel in sterile Petri dishes at 27° C. in the dark. In 2 days roots of the wheat seedlings were large enough to be inoculated (soybean, 2 days; bean, 3 days; tomato, 4 days). Inoculum was prepared from 48-hour cultures on slants of potato-dextrose agar (0.5 per cent dextrose). The bacteria were suspended in sterile tap water, 1 to 2 million cells per cc. In each test, roots of 10 plants were immersed in the

TABLE 1.—*Colony formation by 3 species of plant-pathogenic bacteria on roots of seedlings grown under aseptic conditions*

Bacterial inoculum	Roots[a] on which bacteria developed			
	Wheat	Tomato	Bean	Soybean
Xanthomonas vesicatoria	38/40	11/30	7/10	6/10
Bact. medicaginis var. *phascolicola*	20/30	2/20	19/20	6/10
X. phaseoli var. *sojense*	29/30	4/20	8/10	5/10
No inoculation	1/110	0/50	0/30	2/30

[a] The denominator of the fraction shows the total number of plants of which the roots were inoculated. Since 10 plants were used in each test this figure also gives indirectly the number of tests. The numerator is the number of plants on the roots of which bacterial colonies were present.

inoculum. The inoculated plants were then so placed on sterile moist paper towels in sterile Petri dishes, 3 or 4 plants per dish, that the roots were in the moist air, not in contact with paper or glass. Two days later examination of the roots (mounted in water) with the low power of a microscope showed whether colonies were present. Some of the tests were repeated several times, as shown in table 1.

In all the tests all 3 species of bacteria multiplied and produced colonies on the surface of the roots of wheat. Roots of bean, soybean, and tomato seedlings also supported growth of the bacteria but perhaps not so consistently as wheat (Table 1). In general, there seemed to be no tendency for the bacteria to grow better on the roots of their usual host plants than on the roots of at least one other plant tested. In fact wheat roots seemed to support growth of *Xanthomonas vesicatoria* and *X. phaseoli* var. *sojense* better than did tomato and soybean.

Overwintering in Association with Wheat Roots. The ability of these species of bacteria to overwinter in soil in association with wheat roots was tested. On November 11, 1943, Thorne wheat was sown outdoors in each of 3 small plots, 3 × 5 feet, of spaded nonsterilized soil. Immediately, 750 cc.

of a 6-day beef-peptone-broth culture of one of the 3 species of bacteria was sprinkled on each plot. Each plot was then raked separately with a sterile rake, and sprinkled with 10 liters of tap water. At about monthly intervals between December 11, 1943, and April 14, 1944, ten plants were dug from each plot. The soil was washed from the roots in running tap water. The root system of each plant was crushed separately in a sterile aluminum dish with a sterile glass spatula with 10 cc. tap water, and poured on the lower surface of a water-soaked leaf of the appropriate host: *i.e.*, tomato, soybean, and bean. Development of leaf spots on the inoculated leaves was regarded as evidence of the presence of bacteria on or near the wheat roots. As a further precaution in each test bacteria were isolated from the spots and compared with colonies from the original culture.

Xanthomonas vesicatoria was recovered from wheat roots in December, January, February, and March, but not in April (Table 2). In each test

TABLE 2.—*Recovery of Xanthomonas vesicatoria from roots of wheat plants grown in inoculated soil outdoors in winter of 1943–44 at Lexington, Kentucky. Root systems of 10 plants crushed individually in water and poured on 10 water-soaked tomato leaves*

Date of isolation	No. spots on inoculated leaves
Dec. 11, 1943	2, 0, 7, 15, 0, 0, 3, 12, 1, 0
Jan. 12, 1944	0, 5, 0, 0, 0, 0, 0, 0, 1, 0
Feb. 1, 1944	0, 0, 0, 1, 1, 0, 8, 0, 0, 0
Feb. 23, 1944	27, 90, 75, 100, 80, 0, 0, 0, 2, 3
Mar. 14, 1944	9, 7, 0, 3, 10, 0, 0, 0, 0, 0
Apr. 14, 1944	0, 0, 0, 0, 0, 0, 0, 0, 0, 0

X. vesicatoria was isolated from typical spots on the tomato leaves. Also, in the tests of December, February 23, and March, the isolated bacteria were inoculated onto tomato leaves and produced typical spots. Neither *Bacterium medicaginis* var. *phaseolicola* nor *X. phaseoli* var. *sojense* was recovered from the wheat roots at any time during the period of the test. Although this need not necessarily be interpreted to mean that these organisms cannot overwinter on wheat roots, the general observation that bean halo blight can be avoided by planting disease-free seed may mean that this organism cannot perpetuate itself readily in soil. The tomato and tobacco leaf spots cannot be controlled by planting clean seed; *i.e.*, the organisms exist somewhere else, probably on roots of some nearby plants.

It will be desirable for individual workers to determine the ability of other bacterial pathogens to live and overwinter in association with roots. It would indeed be surprising if the tomato- and tobacco-leaf-spot organisms were unique in this respect. Several or many bacteria may be found to be able to overwinter in association with roots of unrelated species, and perhaps some pathogenic fungi can do likewise—if so, some of the concepts of host range and control of plant diseases by crop rotation may need to be reconsidered.

KENTUCKY AGRICULTURAL EXPERIMENT STATION,
LEXINGTON, KENTUCKY.

LITERATURE CITED

1. DIACHUN, STEPHEN, W. D. VALLEAU, and E. M. JOHNSON. Relation of moisture to invasion of tobacco leaves by *Bacterium tabacum* and *Bacterium angulatum*. Phytopath. 32: 379–387. 1942.
2. ————, ————, and ————. Invasion of water-soaked tobacco leaves by bacteria, solutions, and tobacco mosaic virus. Phytopath. 34: 250–253. 1944.
3. DOOLITTLE, S. P. Tomato diseases. U. S. Dept. Agr. Farmers' Bull. 1934. 1943.
4. HARTER, L. L., and W. J. ZAUMEYER. A monographic study of bean diseases and methods for their control. U. S. Dept. Agr. Technical Bull. 868. 1944.
5. JOHNSON, H. W., and B. KOEHLER. Soybean diseases and their control. U. S. Dept. Agr. Farmers' Bull. 1937. 1943.
6. VALLEAU, W. D., E. M. JOHNSON, and STEPHEN DIACHUN. Angular leafspot and wildfire of tobacco. Ky. Agr. Exp. Bull. 454. 1943.
7. ————, ————, and ————. Root infection of crop plants and weeds by tobacco leaf-spot bacteria. Phytopath. 34: 163–174. 1944.

FAILURE OF BUD AND GRAFT UNIONS OF ROSE INDUCED BY CHALAROPSIS THIELAVIOIDES

KENNETH F. BAKER AND H. EARL THOMAS

(Accepted for publication December 1, 1945)

The "black mold" or "Manetti mold," *Chalaropsis thielavioides* Peyr., has become important in recent years as a cause of graft failure of rose in the east and midwest. The fungus also caused a failure of bud unions in field-grown roses in one area of California during the single year, 1940.

The fungus was perhaps first found on walnut shells from Scotland in 1878 and on apricot roots in Australia in 1902 (5, 9, 11). It was first described (13) as the cause of a root rot of lupine (*Lupinus albus*) in Italy in 1915 and the genus *Chalaropsis* was erected at that time. It was again reported on lupine (*L. albus*) roots in Italy (14) and Germany (12) in 1927. In 1930 it was very damaging to walnut grafts and occurred on carrot roots in England (5). In 1932 the fungus was observed on layered walnuts and on walnut roots in England and it was reported in Palestine in 1933 on peach seedlings from France (5, 9). The fungus was isolated from rose cuttings in Victoria, Australia, by Fish in 1936 (9).

The first definite report of *Chalaropsis thielavioides* in North America seems to have been from Oklahoma in 1934 where it was causing root rot of stored Chinese elm (6). In 1938 the same species caused serious loss of roses in grafting frames in Illinois and New York on rootstocks of *Rosa chinensis* var. *Manetti* from a small area of northern Oregon (8). The disease apparently had been present for some years before discovery (10). In 3 nurseries of Santa Clara County, California, the fungus caused a failure of bud unions on understocks of Manetti, *Rosa odorata,* and *R. multiflora* in 1940 (1); in one field only 26 per cent of the bud inserts had united in 3 successive attempts. It is noteworthy that these losses occurred during the hot, dry months of August and September and that roses budded in a nearby field the following year on cuttings from these infected plants sustained no detectable loss from the disease. In 1945 roses grafted by one grower in Alameda County failed because of decay by this fungus; since it is unusual to graft roses in California, this type of trouble is rare. The disease has not been found in southern California, perhaps because roses there are largely grown on immune Ragged Robin (Gloire des Rosamanes) rootstock and are field-budded rather than grafted. The graft disease also has been observed in Pennsylvania and the fungus found on Washington-grown understocks (19). The graft disease apparently has been associated only with American-grown rootstocks.

The fungus is of importance primarily on greenhouse roses in grafting frames and in nurseries where the plants are budded. Because outdoor roses commonly are propagated on the immune Ragged Robin or resistant *Rosa multiflora* rootstocks, little trouble has been reported or would be expected in that crop.

SYMPTOMS

Usually the disease on rose is first detected by the failure of the bud or scion to unite with the stock. On the interfaces of the bud shield or scion and the rootstock frequently is seen a crust or crumbly mass of black spores and this gives rise to the name "black mold." If, however, the specimens are not observed within a few days the cut surfaces frequently are overrun by secondary fungi which may obscure or obliterate macroscopic evidence of the causal organism; macroconidia may still be observed under the microscope at this stage and afford evidence of the disease.

The earliest stages are frequently overlooked because they are less conspicuous than those outlined above. Very quickly after germination of the

FIG. 1. Failure of Peerless buds to unite with stocks of *Rosa chinensis* var. *Manetti*. Inoculated with a suspension of spores of *Chalaropsis thielavioides* after being budded and tied with grafting rubber; held in cutting frame for 24 days before being photographed. Two stems at left are noninoculated checks showing healthy callus formation. Three stems at right show representative bud failures.

infecting spore there is formed a small area of frosty white endoconidia and scant mycelium which spreads rapidly to cover the cut surfaces. This growth is most conspicuous with high humidity and may be very sparse under dry conditions. On newly infected bud shields and wounded Manetti stems under humid conditions drops of clear liquid may appear for a short time, drying to leave a shiny spot. The frosty white growth darkens within a few days due to color change of the infected tissues and of the fungus itself, and gradually gives way to the black crust of macrospores.

The bud shield is killed and permeated by the fungus. A scion is killed by being cut off from its moisture supply but may not be completely invaded by the fungus. A susceptible type of rootstock may be invaded for a short distance from the cut, but in the immune Ragged Robin only a surface staining has been noted. The fungus does not attack vigorously growing tissue with the rapidity or degree that it does dormant or senescent wood such as bud shields, scions, cuttings, or branch stubs. Cambial activity of such infected tissues is suppressed and no callus is formed.

The graft phase of the disease is well illustrated elsewhere (3, 8, 10). The symptoms of bud failure seen in California are shown in figure 1.

The fungus attacks the basal end of a cutting, preventing formation of callus or roots and sometimes causing its death. Cuttings less seriously affected may show retarded growth of shoots, perhaps because of impaired water absorption through the callus. Leaves cannot be infected (8); that this is not characteristic of chlorophyllous tissue is shown by the susceptibility of green stems of such varieties as IXL and *Rosa multiflora Grifferaie*. Infection of rose roots is known (8) but apparently causes little damage.

The pronounced fruity odor of the fungus on agar media and on rose can be detected in a closed space such as a grafting case; it is said to be due to isobutyl acetate (8).

THE PATHOGEN

The fungus, *Chalaropsis thielavioides*, which caused this disease of rose in California, is identical in cultural and morphological characters with the fungus reported in New York and Oregon (described and illustrated in 8). Also very similar morphologically, but differing somewhat in cultural characters, are the forms from walnut, lupine, Chinese elm, and peach (described and illustrated in 5, 6, 9, 13). The rose fungus has been reported as similar to the imperfect stages of *Ceratostomella radicicola* from date palm (2), *Ceratostomella sp.* from plane trees (17), and *Endoconidiophora variospora* from chestnut oak (4). It was found, however, that the rose fungus did not pair with either strain of *C. radicicola* or *E. variospora* to form perithecia.

All workers are agreed that *Chalaropsis thielavioides* is a wound fungus unable to infect uninjured tissue. The published evidence and our findings are in agreement that the fungus only slightly attacks vigorously growing tissue and produces the most severe damage on dormant parts such as stored Chinese elm roots, rose bud shields, graft scions or cuttings, walnut scions, etc.

The fungus invades the xylem, cortex, vascular rays, and pith of rose and walnut stems, forming macroconidia in the vessels (5, 8).

HOST RANGE AND RESISTANCE[1]

There have been 6 physiologic forms of *Chalaropsis thielavioides* reported; these have been designated as the lupine (13), walnut (5), carrot (5), peach (5), Chinese elm (6), and rose (10) forms. While these forms

[1] Some of the rootstocks used in this work were kindly supplied by Armstrong Nurseries, Ontario; California Nursery Co., Niles; and Western Rose Co., San Fernando.

have been described as differing slightly in cultural and morphological characters, the prime distinction is physiological, resulting in host specificity. That there is, however, physiological uniformity within the species is shown by the fact that the lupine, elm, and rose forms are consistent in vitamin requirement, lacking ability to synthesize thiamine but able to form biotin and pyridoxine, whereas other species differ in these requirements (15).

Tests were made to determine partial host range of some of these forms and of some closely related fungi. The California and New York[2] isolates of the rose form of *Chalaropsis thielavioides* strongly parasitized wounded rootstocks of Manetti, *Rosa odorata*, and *R. multiflora*, but did not infect Ragged Robin. The forms of *C. thielavioides* from lupine,[3] walnut,[4] and Chinese elm,[5] *Ceratostomella radicicola* from date palm,[6] and *Ceratostomella fimbriata* from sweet potato were unable to parasitize any of the 4 rose rootstocks. *Endoconidiophora sp.* from plane tree[7] did not infect Manetti rootstock but was slightly parasitic on Ragged Robin. *Endoconidiophora variospora* from chestnut oak[5] and *Thielaviopsis basicola* from tree peony were nonpathogenic to Manetti and Ragged Robin.

A California isolate of the rose form was tested for pathogenicity on a number of other plants. A freshly peeled area on each stem was inoculated with a pure culture and the stem held in a moist chamber. *Rosa laxa*, once used as an understock, was found to be immune, *R. longicuspis*, *R. nutkana*,[8] and *R. californica*[8] were moderately susceptible, *R. gymnocarpa*, *R. multiflora Grifferaie*, and the rootstock IXL were very susceptible. Stems of orange and *Chaenomeles japonica*, and roots and stems of *Ulmus pumila* were slightly susceptible, stems of pear,[8] sweet cherry, peach, almond, Mariana plum, and Persian and black walnut were moderately susceptible, and apple was immune.

Longrée (8) found that the rose fungus grew slightly on wood of Chinese elm, black walnut, English walnut, poplar, and peach, and on potato tuber and carrot roots in moist chambers. Some growth of the elm form was obtained on wounded rose stems under the same conditions. Hamond (5) reported successful inoculations by walnut and carrot forms on carrot and weak infection on lupine.

Repeated inoculation tests with the California and New York isolates of the rose fungus showed that rootstocks of *Rosa odorata* and Manetti were very susceptible, *R. multiflora* moderately so, and Ragged Robin immune (Fig. 2). This is in line with the observation that the fungus has occurred in rose fields in central California (and presumably in other western states since it occurs on the stocks following digging) where the susceptible

[2] Culture supplied by Dr. L. M. Massey (10).
[3] Culture supplied by Dr. D. E. Bliss from transfer of Peyronel's isolate (13).
[4] Culture supplied by Dr. D. E. Bliss from Centraal Bureau voor Schimmelcultures transfer of Hamond's isolate (5).
[5] Cultures supplied by Dr. R. W. Davidson (4, 6).
[6] Culture supplied by Dr. D. E. Bliss (2).
[7] Culture supplied by Dr. J. M. Walter (17).
[8] Tests with stems from different plants gave inconsistent results indicating variability in resistance.

Manetti, *R. odorata*, and *R. multiflora* rootstocks are used, but has never been observed in southern California where the immune Ragged Robin is used. Use of an immune type of rootstock should prevent the increase of soil inoculum.

A study was made of the immunity of Ragged Robin rootstock. Treatment of hardwood stems in water at 100° C. for 10 minutes killed them and

FIG. 2. Relative resistance of 4 common rose rootstocks to *Chalaropsis thielavioides*. Bark was slit as in budding, stems dipped in spore suspension, and held in cutting frame for 20 days before being photographed. Left to right: Ragged Robin, *Rosa multiflora*, *R. chinensis* var. *Manetti*, and *R. odorata* in increasing order of susceptibility.

destroyed their resistance to the fungus. Similar treatment at 50° C. reduced callusing and sprouting, and enabled the fungus to grow moderately well. Exposure to moisture-saturated air at 50° C. for 10 minutes did not injure the stems and enabled the fungus to develop slightly; loss of immunity did not, then, result from leaching of an inhibitory substance. The susceptibility of stems of Manetti and *Rosa odorata* also was increased by such heating.

The fungus developed quickly in moist chambers on cut surfaces of scions of *Rosa odorata* in contact with Ragged Robin stems, and apparently developed slightly on the latter at points of contact. Resistance is not restricted to phloem and cortical tissues, for a completely peeled Ragged Robin stem is still immune. Wounded stems of *R. odorata* sprayed with a spore suspension and covered with macerated tissue or expressed sap of Ragged Robin stems were not protected from decay.

The addition of sucrose, calcium nitrate, or both to the spore suspensions sprayed onto wounded Ragged Robin stems did not promote infection.

These limited tests indicate that the immunity of Ragged Robin to the rose fungus is restricted to living cells, is decreased by a degree of heat not markedly harmful to the host, and is not due to a strongly diffusible substance.

TEMPERATURE RELATIONS

Studies (5) with the walnut, peach, and carrot forms on carrot agar showed no growth at 0° C., very little at 5°, slight at 12°, optimum at 18–30° (walnut 18–25°, peach 25–30°, and carrot 18°), very little at 33°, and none at 35° C. Longrée (8) found for the rose and elm forms on potato-dextrose agar that the minimum temperature for growth was 0–3.5° C., optimum 18–27.5°, maximum 30.5–33°, and that at 33° C. the fungus was killed in 35 days. The elm fungus caused (20) less root rot of Chinese elm at 1.7–3.3° C. than at 4.4–10.0° or 12.8–23.9° C.

To determine the relation of temperature to growth of the rose fungus on rose, stems of *Rosa odorata* were inoculated and held in moist chambers in the laboratory (about 25° C.) and in refrigerated rooms at 12.8°, 5.0° and 0° C. After 26 days stems at 25° C. were badly decayed; at 12.8° C. stems were blackened for 8 mm. and had abundant endoconidia and macrospores; at 5° C. the stems were covered with endoconidia (and after 79 days the stems were blackened); at 0° C. no fungus development could be seen (after 79 days there was a very sparse endoconidial development and no blackening).

It is apparent that if rose stems as cuttings or stocks are to be stored under conditions inhibitory to the fungus, a temperature as close to 0° C. (21) as practicable without injury to the host should be used. It has been suggested that Chinese elm plants should be stored at 1.7–3.3° C. to control root rot (20).

DISSEMINATION OF THE FUNGUS

The spread of the fungus with infected plant parts has been shown for rose (3, 8), elm (20), and walnut (5). That the endoconidia are adapted to dissemination in water is shown by their gelatinous consistency in mass and by their ease of wetting. The severity of the disease under the moist conditions of the grafting case is circumstantial evidence of water dissemination. Field stocks in California are grown from cuttings which root in place. The soil is pulled away from the crown prior to budding so that the bud may be

inserted close to the roots and thus give a short "neck." The resulting ditch is used for irrigation following budding and the water thus bathes the T cuts and bud shields of an entire row; this supplies an excellent mechanism for rapid spread of the fungus, particularly when irrigations are frequent.

There is strong circumstantial evidence that the fungus may be spread by the worker who does the budding. Because of the abundance and stickiness of the endoconidia it would be expected that they would be spread on the budding knife and the worker's hands or clothing. All of the fields involved in the occurrence of bud failure in Santa Clara County in 1940 had been budded by one worker and the evidence pointed to spread from field to field. Even though the introduced inoculum were scant, the rapid development of endoconidia and the frequent post-budding irrigations would quickly spread the fungus through the field.

The 1940 epidemic in field-budded roses perhaps is best explained by the fact that the cuttings of *Rosa odorata* for one field were buried in clay soil for several weeks after removal from the plants during the rainy season and before being planted in the field. This unusual treatment, necessitated by delayed field preparation, was particularly favorable to development of black mold. The grower reported black crusts on the end of many cuttings on removal from the pit, but apparently there had been no pronounced rooting failure of the cuttings, perhaps because of selection of uninjured stems. It was found (20) that storage of Chinese elm trees in silt loam was more conducive to Chalaropsis root rot than in sand, and that in heavy soil good drainage was required. Applying these facts to the control of the rose disease, the material used for packing rose rootstocks for eastern shipment should be porous and never excessively wet.

Because of the significance of field infection of wounds the soil carryover of the fungus is important. Composite samples were gathered in September, 1941, from the top 2–3 inches of soil in one of the rose fields involved in the 1940 epidemic. This was made up in a heavy aqueous suspension into which peeled Manetti stems were dipped before incubation in a moist chamber. Results were negative for both the bait-sticks and checks. Other composite samples collected from the same field at 3–6-inch depth in October, 1941, were tested similarly and a small number (8 stems in 83) developed *Chalaropsis;* after standing for 6 days the suspension again was tested and 9 in 81 stems were infected. Bits of old stems of *Rosa odorata* from the field were held in moist chambers but no *Chalaropsis* developed; this was to be expected from the rapid replacement of the fungus by more successful saprophytes commonly observed in inoculated stems. There were viable spores present on the stems, however, as shown by rinsing them in water which was bait-tested as above, 1 stem in 101 developing *Chalaropsis;* after the wash water had stood for 7 days a second test gave 2 infected stems in 71. Checks dipped in tap water remained uninfected. Infested sand which had been used in a test in April, 1941, and subsequently held dry in the laboratory, was tested by the same bait method 195 days later. Of 69 stems, 29 devel-

oped *Chalaropsis,* indicating that many spores of the fungus were still alive. The checks remained uninfected. A similar test of the sand after 4 years showed the fungus to be dead. The bait technique should prove useful in determining whether a given field is infested by the fungus.

These tests indicated that the fungus will live over in field soil for nearly a year. It is, therefore, a surprising fact that Manetti cuttings taken in the winter of 1940–41 from the field of stock plants which had sustained such heavy budding losses and planted in a field only a few hundred feet away, had no evidence of the fungus in either stocks, or buds placed in them, during 1941. The disease has not been found since in field-grown roses in California.

CONTROL

Cultural Practices. Since rootstocks for greenhouse roses are grown largely on the west coast and some are grafted by commercial growers in other parts of the country, it is apparent that final control of the graft failure disease will involve cooperation of the growers. It has been stated (10) that "The problem is evidently one to be worked out where the stock is grown and packed. . . . Evidence to date indicates that the problem facing the nurseryman . . . should not be a difficult one." The use of western budded plants instead of locally grafted stock would effectively transfer this problem to the western grower and automatically control the disease. The increasing trend (7) toward budded plants may eventually eliminate losses in grafting frames and reduce the disease to infrequent budding failure in western nurseries. This is regarded as the most effective single control procedure for the disease.[9] The budded type of plant yields as well as the grafted one (18), and can be planted over a longer period of the growing season (7).

A number of other practices would be helpful in reducing losses from this fungus. Cuttings should be planted in the field promptly after being taken from the stock plant. If it is necessary to hold them prior to planting they should be stored at about 0° C., and it would follow that rootstocks shipped east for grafting might be handled in the same way. Should it be necessary to store rootstocks or cuttings in sand or soil, the material should be as porous and well drained as possible; they should never be buried in a pit. It has been shown (21) that dormant rose plants kept best when stored at −1.1° C. in packing material of 36–40 per cent moisture content; no injury from freezing, breaking dormancy, or attack by molds was found under these conditions.

Since the fungus is a wound parasite, freezing and unnecessary mechanical injuries to the plants should be avoided.

Apparently the parasite can be spread by workmen, perhaps on tools, clothing, hands, etc., and careful sanitation is therefore advisable. In situations where the fungus is troublesome it would be a reasonable precaution

[9] Dr. J. A. Milbrath has expressed views in correspondence in agreement with this, and Dr. D. B. Creager has indicated that many Illinois rose growers have discontinued grafting because of the disease.

to have workmen change to clean clothing, wash carefully, and disinfect all tools with 1 per cent formaldehyde (1–40). Similarly all cutting benches, grafting frames, or propagating houses should be sprayed with this solution prior to use. Hamond (5) reported that this procedure plus painting the stems with formaldehyde prior to cutting reduced graft blight of walnut from 95 per cent in 1930 to 7 per cent in 1933. It is necessary to sterilize sand or other material in the grafting frame following a diseased crop before it is again used. This should be done with steam or chloropicrin rather than formaldehyde because of the possibility that the latter may leave a persistent residue.

It would be a wise precaution not to replant roses in an infested field for several years, even though there was no recurrence of the disease in the single case reported here. Rotation of immune Ragged Robin with the susceptible rootstocks would prevent increase of inoculum of the fungus.

The use of rootstocks of Ragged Robin and possibly *Rosa laxa* would aid in reducing losses from this disease. The former stock is commonly used for outdoor roses and might be worthy of consideration for greenhouses.

Propagation by high budding (16) into growing canes of rootstock later made into cuttings for field planting might reduce budding failure. Thus the bud shields would be placed in aerial stems which could hardly have been infested by the water-borne spores of *Chalaropsis*.

Chemical Treatment of Grafts or Buds. Various workers have attempted to inhibit development of *Chalaropsis thielavioides* on various host parts by the use of chemicals. Hamond (5) painted walnut stocks with formaldehyde 1–40 prior to grafting and was fairly successful in eradicating the fungus. Creager (3) used a 2-hour immersion of stock plants in various chemicals before potting; of these potassium permanganate (1 lb. to 25 gals.) and formaldehyde (1–320) were considered best. Wright (20) reported that in tests on chemical treatment of Chinese elm roots no satisfactory control was effected.

Attempts were made to find some fungicidal or fungistatic chemical noninjurious to the host cambium that might be used on rose grafts or buds to control the disease. Stems of Manetti, or less frequently *Rosa odorata* or *R. multiflora,* were used in the tests. Pieces of bark were removed from several (usually 6–12) stems which were then dipped in a suspension of endoconidia and immediately sprayed or dusted with the chemical or dipped in it. The stems were held in moist chambers or in cutting boxes of sand and observed for about a month for presence of the fungus. A few tests with grafted stems held in moist cases, and one limited field test, were made.

Of 74 different materials so tested, in over 110 different dosages and methods of application, only the following had fungicidal or fungistatic properties: Arasan (50 per cent tetramethylthiuramdisulfide—used as dust and in aqueous suspension at 1–200); Arasan plus 1–1000 Isothan Q15 (20 per cent lauryl isoquinolinium bromide); Arasan plus 2 per cent phenyl mercuric acetate in bentonite 1–1; Dithane D–14 (20 per cent disodium ethylene bisdi-

thiocarbamate) 1–100 plus zinc sulfate 1–400 and hydrated lime 1–800; Dow seed disinfectant No. 9 (zinc salt of 2, 4, 5 trichlorophenol); Dow seed disinfectant No. 10 (sodium salt of 2, 4, 5 trichlorophenol); Isothan Q15 1–100 and 1–400; malachite green 1–100; 4 per cent phenyl mercuric chloride in bentonite; sodium dimethyl dithiocarbamate 1–3.3. All of these materials, and many others neither fungicidal nor fungistatic, were injurious to host cambium and prevented its further growth. Attempts to inhibit the development of *Chalaropsis* by simultaneous inoculations of agar cultures and injured rose twigs with *C. thielavioides* and *Penicillium notatum* were unsuccessful.

It would appear to be very difficult to control the graft- or bud-failure diseases by chemical treatment at the point of union.[10] Even though some chemical, inhibitive or lethal to the fungus and noninjurious to host cambium, eventually may be found, for the present control may best be obtained by cultural practices. The use of chemical dips for rootstocks would be advisable prior to grafting operations; formaldehyde 1–320 has been suggested (3). Because of the unimportance of the graft disease in California no studies were made of chemical dips for rootstocks.

SUMMARY

The black mold, *Chalaropsis thielavioides*, caused failure of bud unions in several rose fields, and of grafts in one greenhouse in California. It has not been found in southern California where roses are largely grown on immune Ragged Robin rootstocks.

Infected buds are quickly killed and blackened, and the cut surfaces of scion and stock are blackened and union prevented.

Of the common understocks, *Rosa odorata* and *R. chinensis* var. *Manetti* are very susceptible to the fungus, *R. multiflora* moderately so, and Ragged Robin immune. The immunity factor of Ragged Robin is restricted to living cells and can be decreased by moderate heat treatments. The rose form of the fungus is pathogenic to numerous other roses and woody plants. The lupine, walnut, and Chinese elm forms are nonpathogenic to rose.

The fungus is disseminated by infected plant parts, irrigation water, spattering water drops, soil, and by the worker. It survives in soil or buried plant parts for nearly a year. Infection occurs only through wounds and the fungus is aggressive only on dormant plant parts.

The graft-failure trouble could best be avoided by the use of budded roses, fungus-free rootstocks, sanitation of tools, frames, etc., and chemical treatment of stems prior to grafting. Bud failure is rare, and can be prevented by immediate planting of cuttings or, when necessary, by storing them at 0° C., by rotation with an immune crop, use of immune Ragged Robin rootstocks, by high budding into canes later made into cuttings, and by precaution against spread by workmen.

[10] After this paper was in print a note by J. A. Milbrath appeared (Flor. Rev. 97(2509): 26–27. 1945) in which similar results with 28 fungicides were reported. It was suggested that infected cuttings be soaked 1 hr. in Ceresan (1 lb. per 100 gal. water) before planting, and that benches, tools, etc. be sprayed with copper sulphate (1 lb. per 5 gal. water) prior to use.

Preventive chemical treatments of cut surfaces of the host were unsuccessful because fungicidal and fungistatic materials injured host tissues.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
LOS ANGELES AND BERKELEY.

LITERATURE CITED

1. BAKER, K. F., and H. E. THOMAS. Failure of rose bud-unions caused by *Chalaropsis thielavioides*. Phytopath. **31**: 859. 1941.
2. BLISS, D. E. A new species of Ceratostomella on the date palm. Mycologia **33**: 468–482. 1941.
3. CREAGER, D. B. Control of black mold of rose grafts by chemical treatment. Flor. Rev. **89**(2290): 21–22. 1941.
4. DAVIDSON, R. W. Two American hardwood species of Endoconidiophora described as new. Mycologia **36**: 300–306. 1944.
5. HAMOND, J. B. The morphology, physiology and mode of parasitism of a species of Chalaropsis infecting nursery walnut trees. Journ. Pomol. Hort. Sci. **13**: 81–107. 1935.
6. LAMB, H., E. WRIGHT, and R. W. DAVIDSON. A root rot of Chinese elms. Phytopath. **25**: 652–654. 1935.
7. LAURIE, A., and D. C. KIPLINGER. Culture of greenhouse roses. Ohio Agr. Exp. Sta. Bull. 654. 1944.
8. LONGRÉE, K. *Chalaropsis thielavioides*, cause of 'black mold' of rose grafts. Phytopath. **30**: 793–807. 1940.
9. MASON, E. W. Annotated account of fungi received at the Imperial Mycological Institute, List II, fascicle 3—Special Part: 131–133. Imp. Mycol. Inst., Kew. 1941.
10. MASSEY, L. M., and K. LONGRÉE. Black mold disease of rose grafts. A new rose disease caused by *Chalaropsis thielavioides*. Flor. Exch. **93**(18): 17. 1939.
11. MCALPINE, D. Fungus diseases of stone fruit trees in Australia and their treatment. pp. 125–126, pl. 45. Dept. Agr. Victoria, Australia. 1902.
12. PAPE, H. Krankheiten und Schädlinge der Lupine. Illus. Landw. Zeit. **47**: 316–318. 1927.
13. PEYRONEL, B. Una nuova malattia del Lupino prodotta da *Chalaropsis thielavioides* Peyr. nov. gen. et nova sp. Staz. Sper. Agr. Ital. **49**: 583–596. 1916.
14. PULSELLI, A. Un parassita di alcune specie di Lupinus e di Cytisus. (*Ceratophorum setosum* Kirch. (1892).) Boll. R. Staz. Pat. Veg., n.s., **8**: 50–85. 1928.
15. ROBBINS, W. J., and R. MA. Vitamin deficiencies of twelve fungi. Arch. Biochem. **1**: 219–229. 1942.
16. SCOTT, L. B. Propagating roses by budding in the canes. Proc. Amer. Soc. Hort. Sci. **27**: 467–470. 1930.
17. WALTER, J. M., P. V. MOOK, and C. MAY. Serious disease threatens the sycamore or planetree. Arborist's News **5**: 49–55. 1940.
18. WEINARD, F. F., and S. W. DECKER. Summer-budded versus winter-grafted roses. Ill. Agr. Exp. Sta. Bull. 358. 1930.
19. WEISS, F. Check list revision. Rosa (Rosaceae). U. S. Dept. Agr. Pl. Dis. Reptr. **26**: 206. 1942.
20. WRIGHT, E. Chalaropsis root rot of Chinese elm. Amer. Nurseryman **75**(1): 7–9. 1942.
21. YERKES, G. E., and F. E. GARDNER. Dormant rose plants as affected by temperature and moisture while in storage. Proc. Amer. Soc. Hort. Sci. **32**: 347–350. 1934.

REACTION OF ONION VARIETIES TO YELLOW-DWARF VIRUS AND TO THREE SIMILAR VIRUSES ISOLATED FROM SHALLOT, GARLIC, AND NARCISSUS

PHILIP BRIERLEY[1] AND FLOYD F. SMITH[2]

(Accepted for publication December 5, 1945)

As a part of a study of the nature and relationships of viruses isolated from shallot (*Allium ascalonicum* L.), garlic (*A. sativum* L.), and narcissus (*Narcissus pseudonarcissus* L. var. King Alfred), 30 varieties or lots of onions (*A. cepa* L.) were inoculated with these viruses and with an isolate of onion-yellow-dwarf virus (5, 6, 7) (= Allium virus 1 K. M. Smith = *Marmor cepae* Holmes) obtained from potato onion (*A. cepa* var. *solanium* Alef.) from West Virginia in 1943.

The last named is considered to be the common or type strain of yellow-dwarf virus, namely, that originally described by Melhus *et al.* (6). Henderson (4), who quotes Giddings as stating that this disease was confined to potato onions when observed in West Virginia in 1916, considered a sample of the West Virginia virus a duplicate of the one under study in Iowa. Other isolates from onions by the writers, namely, one from New York from perennial tree onion (*A. cepa* var. *viviparum* Metz) supplied by H. A. Jones and two from Oregon, from commercial onions supplied by F. P. McWhorter, appear to be identical with the West Virginia virus. This admittedly small number of samples indicates that yellow-dwarf occurring in commercial onions is usually of this type, and that other types such as our isolates from shallot, garlic, and narcissus are at least not commonly encountered in onions in nature. An isolate from Everready onions received from England in 1944 by H. A. Jones also appears to be of the common yellow-dwarf type, agreeing with this in symptom expression in the test variety Ebenezer, and failing to infect Utah Sweet Spanish.

The shallot virus was isolated from commercial shallot supplied from Baton Rouge, Louisiana, by J. E. Welch in 1943. Of 13 other samples supplied by E. C. Tims from Louisiana, 10 were found infected with yellow-dwarf as shown by symptoms and by transfer to onion. In one sample of a strong-growing variety marked "Miller No. 1" only 2 of 16 plants were affected, and a virus-free line of this has been segregated. Symptoms in shallot are essentially as in onion, and readily recognizable, but Louisiana shallots are apparently acceptable commercially when affected although Green (2) has shown that yield is markedly reduced by a disease very similar to this in England. In onions the symptoms of the shallot virus are similar to those of common yellow-dwarf, except for greater virulence

[1] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering.

[2] Entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland.

as shown by more extreme dwarfing and folding downward of leaves. The shallot virus is probably a strain of yellow-dwarf, but comparisons of properties and other criteria are not yet complete. Only the one isolate from shallot has been compared in detail with yellow-dwarf, and it is not certain that the virulent strain is characteristic of shallot infections. This virus is readily transmitted from shallot to onion by mechanical inoculation and also by *Myzus persicae* Sulz. with the methods used for viruses that are nonpersistent in the vector.

The garlic virus was isolated from commercial late or Italian garlic submitted by F. P. McWhorter from Oregon in 1943. Two other samples of late garlic from Oregon and one sample of early or Mexican garlic and one of late garlic from California expressed 100 per cent mosaic symptoms when grown in our greenhouse. Indeed, all *Allium sativum* garlic seen by the writers has shown mosaic. Transfers from several garlic samples to onion have been accomplished by mechanical inoculation and by *Myzus persicae*, using the transfer technique adapted to viruses nonpersistent in the vector, but these transfers typically yield low percentages of infection. These isolates, including one from garlic collected in Lanchow, China, by T. P. Dykstra, appear to be similar in the test onion variety Ebenezer, but only one has been compared with yellow-dwarf in detail. Symptoms in onions are similar to yellow-dwarf symptoms in early stages, but there is much less folding down of the leaves in later stages and the disease is much milder in effect. F. P. McWhorter[3] recalls collecting a mild disease of this order in onions in Oregon, but we have no further record of such a disease appearing naturally in onions. The status of the garlic virus is still uncertain, but it may prove to be a mild strain of onion-yellow-dwarf virus.

The narcissus isolate was made to Ebenezer onion from typical narcissus mosaic material supplied by F. A. Haasis. Transfer of this by *Myzus persicae* has succeeded only once in several attempts and the virus has not yet been returned to narcissus. Therefore this isolate is not necessarily narcissus-mosaic virus (3) nor necessarily a common invader of narcissus. No transfer from narcissus to onion by mechanical means has succeeded even under conditions that permit success with the garlic to onion transfer. Symptoms of the narcissus isolate in onions are similar to those of garlic mosaic, and the virus is evidently closely allied to the latter.

All virus cultures were maintained in Ebenezer onions, the four isolates being carried in parallel so that inoculum was of comparable age for each. In the writers' earlier tests, inoculation by juice and by *Myzus persicae* transfer produced similar results, varieties susceptible by one method proving susceptible by the other method also. Inasmuch as all four isolates are transferred with ease from onion to onion by the carborundum-leaf-rubbing method, this method was used in the variety tests here reported.

Onion varieties for testing were grown from seed supplied by H. A. Jones and planted September 21, 1944, except that two additional lots of Yellow

[3] Personal communication.

Sweet Spanish and one of Utah Sweet Spanish were sown February 16, 1945, to confirm unexpected findings with the main plantings. Inoculations were begun in January and continued into May, or until infection with one or both of the more virulent viruses was complete. Some of the September-planted onions were inoculated six times, those planted in February three times.

TABLE 1.—*Reaction of onion varieties to yellow-dwarf virus and to three other isolates from shallot, garlic, and narcissus*

Variety	Previous reaction[a] to yellow-dwarf		No. plants infected[b] with virus strain from:			
	Beltsville	Iowa	Yellow-dwarf	Shallot	Garlic	Narcissus
Australian Brown	S	S	10	10	7	6
Brigham Yellow Globe	S		10	10	10	10
California Early Red	S		7	10	7	6
Creole	S	S	5	10	3	2
Early Yellow Globe	S		10	10	8	9
Ebenezer	S		10	10	9	10
Golden Globe	S		10	10	10	9
Italian Red			8	10	10	9
Michigan Yellow Globe	S	S	10	10	8	9
Red Wethersfield	S	S	10	10	4	9
Southport Red Globe	S		10	10	6	8
Southport White Globe	S		10	10	9	6
Stockton Yellow Globe	S		7	10	4	4
White Portugal	S		10	10	8	8
Yellow Globe Danvers	S	S	10	10	6	6
Yellow Globe Danvers	S	S	10	10	10	9
Crystal Grano	I or S		10	10	8	4
Early Yellow Babosa			0	10	0	0
White Babosa			0	10	0	0
Utah Sweet Spanish	I	(Riverside Sweet Spanish, I)	0	9	0	0
White Sweet Spanish[c]	I		2	10	2	0
Yellow Sweet Spanish (1943 seed)[c]	I		6	10	4	7
Yellow Sweet Spanish (1943 seed)[c]	I		7	10	2	2
Yellow Sweet Spanish (1944 seed)	I		0	9	0	0
Crystal Wax	I	S	0	10	0	0
Lord Howe Island	I		0	10	0	0
San Joaquin	I		0	10	0	0
Yellow Bermuda	I	(Bermuda Red, S)	0	10	0	0
Amphidiploid var. Beltsville	I		0	0[d]	0	0
Nebuka	I	I	0	0[d]	0	0

[a] S = Susceptible, I = Immune.
[b] In each case 10 plants were inoculated.
[c] Strain mixture in seed lot.
[d] Subinoculations also negative.

The result of inoculating the four viruses into 27 varieties of onions and three duplicate lots is shown in table 1. It is evident that 10 of the 30 lots showed immunity to typical yellow-dwarf virus, and that all lots showing immunity to this also proved immune to the milder viruses from garlic and narcissus. In strong contrast, the shallot isolate infected all typical onion varieties exposed, and only Nebuka (*Allium fistulosum* L.) and an amphi-

diploid (*A. fistulosum* × *A. cepa*) proved immune to this virus. Nebuka is a nonbulbing species, used extensively in the Orient as a green onion. The amphidiploid tested here was from a hybrid of Nebuka by White Portugal onion, produced by H. A. Jones and associates, and about to be introduced as a green bunching onion under the varietal name Beltsville.

Henderson (4) reported only Riverside Sweet Spanish immune from yellow-dwarf among 36 onion varieties tested in Iowa, but also listed Nebuka as immune, not regarding this as an onion. Onion varieties have changed during the intervening 10 years, so that comparatively few of those listed by Henderson are included in our trials. Henderson lists Crystal White Wax and Bermuda Red as susceptible to yellow-dwarf. Crystal Wax and Yellow Bermuda as now carried by H. A. Jones have proved uniformly immune from this virus in our tests. It is not reasonable to assume that the Iowa outbreak was of the shallot type, for this strain readily infects all Sweet Spanish onions we have tested. Additional varieties susceptible to yellow-dwarf in Henderson's list and also in other trials at the Plant Industry Station, Beltsville, Maryland, are Bottle onion (= Scott County Globe), Mountain Danvers, Potato onion, and Southport Yellow Globe. The White Persian variety, not tested in Iowa, proved susceptible in our trials.

Several discrepancies appear in our own data for the Grano and Sweet Spanish varieties. In table 1 Crystal Grano appears fully susceptible to yellow-dwarf, and the related Babosa varieties immune. In previous trials two lots of Early Grano and one of Crystal Grano gave immune reactions, but one of Crystal Grano yielded 54 per cent susceptible, with the remainder insusceptible after five inoculations.

Sweet Spanish varieties have proved uniformly immune from yellow-dwarf in 18 previous trials, and Utah Sweet Spanish withstood repeated inoculation in a parallel experiment (1). In the present series (Table 1) one lot of Yellow Sweet Spanish and one of White Sweet Spanish yielded susceptibles, not only to the common yellow-dwarf virus but also to the milder viruses from garlic and narcissus. This Yellow Sweet Spanish from 1943 seed was replanted in February together with 1944 seed of this variety and seed of Utah Sweet Spanish for comparison. In this second trial of Yellow Sweet Spanish from 1943 seed, susceptibles again appeared, while the 1944 seed lot of this variety and the Utah variety yielded only immunes. The 1943 seed lot gave rise to nonglossy individuals as well as to semiglossy plants characteristic of Sweet Spanish types. It is therefore considered that this Yellow Sweet Spanish was a strain mixture not characteristic of the variety. A similar mixture may also be assumed to have been present in the White Sweet Spanish lot listed in table 1.

Inasmuch as commercial onion varieties are thus found to vary in reaction it is preferable to state that lines immune from yellow-dwarf have been found to occur in the Spanish-type varieties Burrell's Sweet Spanish, Colorado No. 6, Utah Sweet Spanish, White Sweet Spanish, and Yellow Sweet

Spanish, and also in Crystal Grano, Early Grano, Early Yellow Babosa, White Babosa, Crystal Wax, Lord Howe Island, San Joaquin, and Yellow Bermuda. It should be emphasized that all these lines that have been tested have proved susceptible to the shallot virus, only Nebuka and the Nebuka × White Portugal amphidiploid variety Beltsville proving immune to the latter.

A study of the inheritance of immunity from yellow-dwarf, carried on through three seasons, has failed to provide a satisfactory genetic interpretation. It seems certain that immunity from typical yellow-dwarf could be incorporated into all onion varieties, but it is highly unlikely that the inheritance of immunity will prove simple enough to permit the breeder to proceed without repeated testing for yellow-dwarf reaction in successive progenies. Extensive series of subinoculations from inoculated plants in these studies indicate that symptom expression is reliable in all varieties provided that they are maintained in active vegetative growth.

It should be stated that shallots are grown commercially in the United States only in Louisiana. If these plants are brought into commercial onion districts for horticultural or genetic studies, it would be wise to start them from seed, in order to avoid the danger of extending the range of the shallot virus.

SUMMARY

Twenty-seven varieties of onions were mechanically inoculated with onion-yellow-dwarf virus and with related isolates from shallot, garlic, and narcissus. Ten variety samples proved immune from yellow-dwarf and also to the isolates from garlic and narcissus, while 17 varieties were susceptible to these three viruses.

All but Nebuka (*Allium fistulosum*) and an amphidiploid, Nebuka × White Portugal, proved susceptible to the shallot virus. These two green-bunching types are immune to all four viruses under test.

All four viruses were transmissible by *Myzus persicae*.

PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

LITERATURE CITED

1. BRIERLEY, P., and N. W. STUART. Influence of nitrogen nutrition on susceptibility of onions to yellow-dwarf virus. Phytopath. **36**: 297–301. 1946.
2. GREEN, D. E. A suspected virus disease of shallots and onions. Jour. Roy. Hort. Soc. **70**: 24–29. 1945.
3. HAASIS, F. A. Studies on narcissus mosaic. Cornell Agr. Exp. Stat. Memoir 224. 1939.
4. HENDERSON, W. J. Yellow dwarf, a virus disease of onions, and its control. Iowa Agr. Exp. Stat. Research Bul. 188. 1935.
5. HOLMES, F. O. Handbook of phytopathogenic viruses. 221 pp. Minneapolis. 1939.
6. MELHUS, I. E., C. S. REDDY, W. J. HENDERSON, and E. VESTAL. A new virus disease epidemic on onions. Phytopath. **19**: 73–77. 1929.
7. SMITH, K. M. A textbook of plant virus diseases. 615 pp. London. 1937.

INFLUENCE OF NITROGEN NUTRITION ON SUSCEPTIBILITY OF ONIONS TO YELLOW-DWARF VIRUS

PHILIP BRIERLEY[1] AND NEIL W. STUART[2]

(Accepted for publication December 5, 1945)

In testing onion (*Allium cepa* L.) varieties and hybrids for reaction to onion-yellow-dwarf virus[3, 4, 5] (= Allium virus 1 K. M. Smith = *Marmor cepae* Holmes) it was noted that low infection was associated with low nitrogen level in certain potting soils. In the season of 1944–45 a test of this apparent relation was made by growing 4 varieties of onions at high and low nitrogen levels in subirrigated benches filled with Haydite.

Varieties. Seed of 4 commercial varieties was furnished by H. A. Jones. Ebenezer was selected as a representative of the highly susceptible class, and Utah Sweet Spanish as an immune variety. Two varieties, Creole and Stockton Yellow Globe, were chosen as apparently resistant to but not immune from this virus, on the basis of previous testing in soil culture.

Source of virus. Onion-yellow-dwarf virus was isolated from naturally infected Multiplier onion (*Allium cepa* var. *solanium* Alef.) from West Virginia in March, 1943, and subcultured in Ebenezer. All inoculations were made by the carborundum-leaf-rubbing method, which has proved highly efficient for transfer of this virus under favorable test conditions.

Experimental design. Eight of nine cement bench sections, each 8 feet by 57 inches by 7 inches were filled with "B" grade Haydite, and the ninth with a good soil mixture, composed of 2 parts composted soil, 2 parts sand, 1 part manure, and 1 part muck. Seeds of 3 varieties were sown August 16, 1944, directly in these benches. Seeds of the fourth variety, Creole, were sown on the same date in thumb pots of the same two media, and shaded 2 hours daily with black sateen cloth until September 21 to avoid premature bulbing likely to occur in this variety under long-day illumination. The Creole plants were transplanted into the assigned bench sections on September 26. Four replications of high nitrogen level and 4 of low nitrogen level were randomized among the 8 Haydite-filled bench sections. In each of these 8 plots and the one soil plot 3 rows of each of the 4 onion varieties were grown, with plant counts per variety plot ranging from 37 to 64. The total number of onions in the experiment was 2,033, with a mean of 56.5 plants per variety plot.

All plants were watered from the top until August 30. At this date one-half the final nutrient level was supplied to each Haydite plot by pumping from the tanks twice daily.

[1] Pathologist, and [2] Physiologist, both of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland.

[3] Melhus, I. E., C. S. Reddy, W. J. Henderson, and E. Vestal. A new virus disease epidemic on onions. Phytopath. 19: 73–77. 1929.

[4] Smith, K. M. A text-book of plant virus diseases. 615 pp. London. 1937.

[5] Holmes, F. O. Handbook of phytopathogenic viruses. 221 pp. Minneapolis. 1939.

Each tank had a volume of 198 liters of solution. On September 27, solutions were changed and full-strength nutrient levels were then maintained. Solutions were completely renewed at intervals of 4 weeks. At weekly intervals the nitrate content and ammonia nitrogen content were determined and restored to previous levels. Nitrate was determined by the phenoldisulphonic acid method using an Aminco Type F photometer. Ammonia was measured in the same instrument after nesslerization.

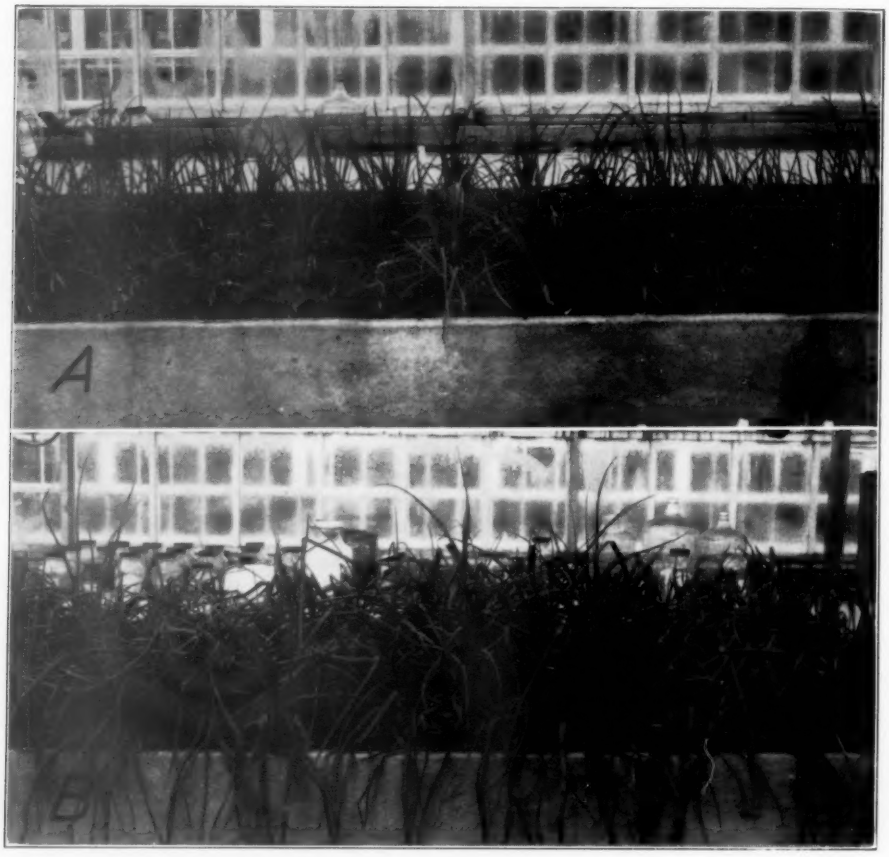


FIG. 1. Characteristics of growth of onions in Haydite culture December 6, 1944: A at low N level (6 ppm.); B at high N level (60 ppm.). Note that growth is normal at the low level, but much more vigorous at the higher level of nitrogen.

The low N nutrient solution contained the following millimolecular concentrations of six nutrient salts: 0.18 Ca $(NO_3)_2 \cdot 4\,H_2O$ [5 ppm. N and 7.2 ppm. Ca], 0.036 $(NH_4)_2SO_4$ [1 ppm. N], 0.75 K_2SO_4 [58.6 ppm. K], 1.62 $CaCl_2 \cdot 2\,H_2O$ [64.8 ppm. Ca], 1.0 $MgSO_4 \cdot 7\,H_2O$ [24.3 ppm. Mg], and 1.0 KH_2PO_4 [31 ppm. P and 39.1 ppm. K]. The high N nutrient solution differed in that it contained 10 times as much $Ca(NO_3)_2 \cdot 4\,H_2O$ and $(NH_4)_2SO_4$ but contained no $CaCl_2 \cdot 2\,H_2O$. Both low and high N solu-

tions also contained 2 ppm. of Fe, 1.5 of Mn, 0.5 of B, 0.15 of Zn, 0.06 of Cu, and 1 cc. per liter of approximately 0.1 N H_2SO_4.

The high nitrogen level was chosen as favorable for vigorous vegetative growth of onions on the basis of previous studies of onion-seed production in Haydite culture. The low nitrogen level was chosen arbitrarily at one-tenth the high level, providing a solution capable of supporting less succulent but still fairly normal growth without extreme symptoms of nitrogen deficiency (Fig. 1). During the weekly intervals between analysis and restoration of solution levels, all the ammonia nitrogen was commonly absorbed or oxidized at both levels. The nitrate nitrogen sometimes dropped as low as 25 to 30 ppm. at the higher level and 2 to 3 ppm. at the lower level, but the proportional relation of 10 to 1 remained nearly constant.

Results. All plants were inoculated with a uniform preparation of yellow-dwarf-virus extract on October 24, and again on November 14. First symptoms were recognized in Ebenezer, Creole, and Stockton Yellow Globe

TABLE 1.—*Effect of high and low nitrogen levels in Haydite culture on susceptibility of onions to yellow dwarf, expressed as percentage infection. Means of 4 replicates compared with soil culture not replicated*

Variety	Haydite culture				Soil culture
	High N, Dec. 5 (Col. 1)	Low N, Dec. 5 (Col. 2)	Means of columns 1 and 2	Low N to Dec. 5, High N to Jan. 3	Dec. 5
Ebenezer	99.7	86.3	93.0[a]	87.6[a]	96.9
Stockton Yellow Globe	41.6	20.3	30.8	27.7	40.0
Creole	40.2	17.9	29.0	25.5	30.0
Mean of 3 vars.	60.4[b]	41.5	50.9	47.0	56.5
Utah Sweet Spanish	0.0	0.0	0.0	.....	0.0

[a] Differs significantly (odds 99 : 1) from means of other varieties.
[b] Differs significantly (odds 99 : 1) from means of other treatments.

at the high N level and in soil on November 3, and at the low N level on November 7. On December 5 all recognizably diseased plants were removed from the beds and scored. The mean percentage infection for varieties and N levels is shown in table 1.

Variance analysis of the data for percentage infection (Table 1, Columns 1 and 2) on December 5 shows differences in yellow dwarf expression at the 1 per cent level between varieties and between N levels, but no significant interaction. Higher percentages of infection at the high N level than at the low level are significant within the resistant varieties Stockton Yellow Globe and Creole; differences are smaller and nonsignificant within the susceptible variety Ebenezer. The mean differences between the susceptible Ebenezer and the resistant varieties, Stockton Yellow Globe and Creole, are very highly significant, but there is no significant difference between the two resistant varieties. The plot of good soil closely approached the high N level plots in performance. The immune Utah Sweet Spanish was unaffected at either level of N. It is concluded that low N levels may significantly

hamper expression of yellow dwarf, that this effect is more likely to be encountered in varieties having a tendency merely to resist infection, but that nutrition levels show no tendency to break down immunity.

At this point it seemed of interest to determine whether the low infection observed at low N level represented an actually lower percentage infection or merely a failure to express symptoms. Accordingly, the low N level plots were raised to high N level on December 5, and so maintained without further inoculation until January 3. During this month at high N level 36 additional plants in those formerly low N level plots showed infection, a mean gain of 5.5 ± 2.03 per cent per variety plot, showing that additional plants previously infected were able to express symptoms at the high N level.

Variance analysis of the data for infection in low N plots January 3, one month after these were raised to high N level, in comparison with the December 5 data for high N level (Table 1, Column 1 vs. Column 4) shows the same general differences as the first analysis. Therefore a real difference is found between varieties and between N levels with respect to total infection, including infections that were not yet evident on December 5 at the low N level. Differences due to N level and to variety are thus highly significant with respect to percentage infection as well as to expression of symptoms.

The retarding or preventive effect of low N level is greatest, and highly significant, with respect to infection, but small and nonsignificant with respect to delay of expression (Table 1, Column 2 vs. Column 4). Admittedly, it was not determined whether further expression would have occurred in an additional month at low N level, or whether further expression would have occurred in the high N level plots without further inoculation, but such refinements could not have altered the conclusion that low N level depresses infection.

In commercial culture of onions, nitrogen will not often fall low enough to affect infection with yellow dwarf. However, in experimental pot culture in soil, nitrogen may become limiting and lead to low infection and expression. It is important, therefore, to maintain satisfactory N levels in such cultures when studying yellow dwarf effects.

RESISTANCE TO ONION YELLOW DWARF

Immunity to yellow dwarf in some Sweet Spanish varieties has long been known[6] but the resistance of Creole and Stockton Yellow Globe has not been noted previously. Resistance is of minor interest to the breeder of new types when immunity is available, except that it may act as a confusing factor in interpreting inheritance of immunity. The present experiment provides evidence that these resistant onions differ sharply in response to yellow dwarf inoculation from the highly susceptible Ebenezer and also from the immune Utah Sweet Spanish.

[6] Henderson, W. J. Yellow dwarf, a virus disease of onions, and its control. Iowa Agr. Exp. Stat. Res. Bul. 188. 1935.

It was considered desirable to determine whether a resistant variety is genetically uniform, so that all individuals are capable of infection, or whether evidence of segregation for immunity could be found. Accordingly all varieties in high N level plots were continued, and plants in the soil plot were transplanted to these on December 5 to increase the size of samples. These plots were maintained at high N level until June and inoculated 17 times in all, at intervals up to April 17, when bulbing and flowering tendencies made further inoculations unprofitable. The cumulative percentages of infection recorded at intervals are shown in table 2.

TABLE 2.—*Cumulative percentage of yellow dwarf infection in 4 varieties of onions grown at high N level in Haydite, resulting from 17 successive inoculations between October 24 and April 17*

Variety	Original number of plants	Cumulative percentage infection							
		Nov. 14	Dec. 5	Jan. 23	Feb. 27	Mar. 27	Apr. 17	May 15	June 11
Ebenezer	299	94.3	99.0	100.0	.....	.....	.....	.....	.....
Stockton Yellow Globe	261	30.3	41.4	70.5	80.1	88.1	92.3	92.7	93.1
Creole	296	18.2	37.8	58.4	68.6	79.7	83.8	83.8	83.8
Utah Sweet Spanish	289	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The susceptible Ebenezer develops a high percentage infection promptly and the few escapes soon succumb to further inoculation. In the resistant Stockton Yellow Globe and Creole varieties 20 to 30 per cent are infected early, and the escapes gradually are infected in small rather uniform increments over several months and many exposures. It was not feasible in this study to demonstrate that all individuals of the Stockton Yellow Globe and Creole varieties are susceptible, but this seems more probable than that they show segregation for immunity.

It is also of interest to note that there was no breakdown of immunity in Utah Sweet Spanish at favorable high nitrogen level and under repeated exposure.

SUMMARY

Four varieties of onions grown at high (60 ppm.) and low (6 ppm.) weekly initial N levels in Haydite were inoculated with onion-yellow-dwarf virus. Both the percentage expression of symptoms and the percentage actual infection were lower at the lower N level, both differences being highly significant.

Immunity of the Utah Sweet Spanish variety was unaffected by repeated inoculation at a high N level that enhanced infection in both susceptible and resistant varieties. Two varieties, Creole and Stockton Yellow Globe, showed resistance to infection distinct from the immunity of Utah Sweet Spanish.

PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND.

THE RELATION OF SOIL TEMPERATURE TO THE DEVELOPMENT OF ARMILLARIA ROOT ROT[1]

DONALD E. BLISS[2]

(Accepted for publication December 7, 1945)

Soil temperature is one of the important conditioning factors in the development of root diseases. Much of the literature on this subject has been summarized by Jones *et al.* (7), and, more recently, by Garrett (6). The relation of soil environment to the development of Armillaria root rot has, however, received little attention, though the causal agent, *Armillaria mellea* (Vahl) Quel., has been known for many years and the disease is of considerable economic importance. A series of experiments was undertaken to determine, if possible, the effect of soil temperatures on the growth of certain economic and ornamental species of plants, and on the development of Armillaria root rot on these plants.

The effects of soil temperature on plant growth are best studied under controlled conditions free from the diurnal and seasonal fluctuations of temperature characteristic of the natural soil environment. Modern thermostatically controlled equipment provides a practical means of obtaining nearly constant temperatures in soil for extended periods and over a comparatively wide range. The results of such experiments help to explain the geographic distribution of the disease and its seasonal development. Although the temperature of soil in nature cannot usually be controlled, exploratory studies of this kind serve as a guide in formulating effective control measures. Some of the cardinal findings of the first of the experiments have been given in a brief preliminary report (1). A complete, more detailed report is presented here.

MATERIALS AND METHODS

The effect of soil temperature on plant growth and disease development was studied in the greenhouse by means of five soil-temperature tanks (2, see fig. 22, p. 61), in which water baths were maintained at controlled temperatures. Seedlings or cuttings were grown in light sandy loam in metal containers, or pots, 8 inches in diameter and 12 inches tall, without holes for drainage. These metal pots were inserted through holes in the tank lids and submerged to a depth of 8.5 inches in the water baths. There were 8 metal pots in each soil-temperature tank.

Nine species of plants were used: Koethen and Homosassa sweet orange (*Citrus sinensis* [L.] Osbeck), Standard sour orange (*C. aurantium* L.),

[1] Paper No. 536, University of California Citrus Experiment Station, Riverside, California.

[2] Associate Plant Pathologist in the Experiment Station. The writer is indebted to H. S. Faweett and A. R. C. Haas for helpful advice; to L. C. Cochran, A. S. Rhoads, and J. M. Wallace for plant materials; and to Robert O. Bream, Newton Noble, Carl Moore, Robert J. Drake, George Jeffrey, Elizabeth Parker, Pearl Golding, L. C. Masters, L. L. Huillier, and Carol McFarland for assistance in the laboratory and greenhouse.

Sampson tangelo (hybrid of *C. paradisi* Macf. and *C. reticulata* Blanco), California pepper tree (*Schinus molle* L.), casuarina (*Casuarina stricta* Dry.), Lovell peach (*Prunus persica* Sieb. and Zucc.), Royal apricot (*P. armeniaca* L.), geranium (*Pelargonium hortorum* Bailey), and Ragged Robin rose (*Rosa sp.*, var. Gloire des Rosomanes).

The citrus seedlings of experiments 1 and 6 (Table 1) were transplanted to small pots after one year in the seedbed, and were later transferred to the metal containers for the soil-temperature tanks. Stratified seeds of Lovell peach and of Royal apricot (experiment 2) were germinated in flats and transplanted to the metal pots. After one year's growth under uniform

TABLE 1.—*Temperatures of air and of water baths in greenhouse soil-temperature-tank experiments with Armillaria root rot*

Number, beginning date, and length of experiment	Air temperature in greenhouse, range	Water-bath temperature					Suscept	Plants in each tank	
		Tank I	Tank II	Tank III	Tank IV	Tank V		Non-inoculated	Inoculated
	°C.	°C.	°C.	°C.	°C.	°C.		*No.*	*No.*
1. Nov. 3, 1938; 438 days	16.0–30.0	10	17	24	31	38	Koethen sweet orange	3	9
							Standard sour orange	3	9
2. Jan. 15, 1940; 218 days	21.0–27.0	10	17	24	31	38	Lovell peach	3	9
							Royal apricot	3	3
							Geranium	3	3
3. Mar. 31, 1941; 240 days	20.0–27.8	10	15	20	25	30	California pepper tree	6	14
4. Nov. 27, 1941; 181 days	21.0–24.4	8	13	18	23	28	Ragged Robin rose	2	6
5. Sept. 14, 1942; 324 days	16.7–26.7	7	12	17	22	27	Casuarina	4	6
6. Sept. 22, 1943; 385 days	16.1–23.9	7	12	17	22	27	Homosassa sweet orange	2	6
							Sampson tangelo	2	6

conditions, these plants were placed in cold storage for one month, before the beginning of the soil-temperature experiment, to induce dormancy. The geraniums (experiment 2) and Ragged Robin roses (experiment 4) were grown from cuttings. Seedlings of California pepper tree (experiment 3) and of casuarina (experiment 5) were first transplanted from flats to small pots and thence to the metal containers for the temperature tanks.

Each metal pot contained 1 to 3 plants, and weighed amounts of air-dried potting soil and water. When prepared, all containers in any one experiment had the same total weight and nearly uniform soil-moisture percentages. The soil was irrigated with distilled water to avoid excessive accumulation of salts. The soil-moisture content was maintained within the range between wilting percentage and field capacity by adding water at 2- to 7-day intervals to raise the total weight of each pot to a certain predetermined level. Small amounts of calcium nitrate or ammonium sulphate were applied

uniformly to all pots in any one experiment to maintain an adequate nitrogen supply. All plants were grown at first on a bench in the greenhouse under similar environmental conditions, before the beginning of the differential soil-temperature treatments. The plants were generally vigorous and reasonably uniform in size at the beginning of each experiment.

Temperature Control

The water in the five soil-temperature tanks was heated by electric soil-heating cable; that in three tanks was also cooled by means of electric refrigeration and circulated by means of pumps. After the plants had become established, the metal containers in which they were growing were inserted through the holes in the tank lids and partially submerged in the water baths. Except in experiment 1, a 2-inch layer of dry, granulated peat moss or cork was spread over the soil of each container for insulation immediately after inoculation. The soil at the surface, though reasonably well protected, was affected somewhat by the air temperature, especially if the temperature gradient was large. Differences up to 2° C. were noted between temperatures of the water baths and of soil in the surface-inch of submerged pots.

The temperatures of the air and of the water baths were recorded each day at 8 : 30 a.m., and at 1 : 00 and 5 : 00 p.m., to check the accuracy of temperature control. By means of thermostatically controlled steam coils and a desert cooler, the air temperatures of the greenhouse were maintained mostly between 21° and 27° C. The greenhouse was covered in summer with a coat of white paint to lower the air temperature and reduce radiation. The temperatures of the water baths ranged mostly within $\pm 0.5°$ of the desired levels. Weekly mean temperatures of the air were calculated from thermograph charts by means of a polar planimeter (4, p. 56). Mean temperatures for each water bath were obtained by averaging 17 to 21 readings per week. With few exceptions, the performance of the control mechanism was satisfactory. The weekly mean temperatures of the water baths showed comparatively small ranges, the minus variation being never more than 0.9° and the plus variation being from 0.1° to 2.5°.

Inoculation

Stock culture B–508 of *Armillaria mellea* was used in the preparation of all inoculum for experiments 1 to 5, inclusive. This culture had been isolated October 22, 1937, from the sour-orange rootstock of a mature Valencia-orange tree near Azusa, California. Two strains of *A. mellea* were used in experiment 6: culture B–700, a reisolate of B–508, and culture B–701, a reisolate of B–677, which had been isolated July 3, 1941, from a peach root collected at Hamlet, North Carolina, and sent to me through the courtesy of Dr. J. S. Cooley.

Segments of citrus roots or pepper-tree stems, 1 to 2 inches in diameter and 7 inches long, were used in the preparation of the inoculum. The seg-

ments were sterilized in an autoclave, inoculated with *Armillaria,* and incubated at 20° C. until thoroughly invaded by mycelium and covered with the pseudosclerotium of the fungus. The segments were then inserted singly in the pots, in vertical holes made in the soil with a soil tube. Inoculation was done with the least possible disturbance of the soil or injury to the roots. Control plants were treated in the same manner but with sterilized root segments. The sterile segments used in the control pots in experiments 2, 3, and 4, had been previously inoculated with the pathogen. The inoculations, which were made a few days after the beginning of the differential temperature treatments, marked the beginning of the experiments (Table 1).

RESULTS

Effect of Soil Temperature on the Noninoculated Suscepts

In experiment 1, the growth of Koethen-sweet-orange and Standard-sour-orange seedlings was greatly retarded at 10° C., and the new leaves were small and very chlorotic. Greatest top growth occurred at 31°, and the roots grew most rapidly at 17° to 31°. In experiment 6, all Homosassa-sweet-orange seedlings and Sampson-tangelo seedlings died at 7°; those at 12° were chlorotic and subnormal. Root development was similar to that of the tops, 12° being near the critical temperature for growth, and 17° to 27° being a favorable range. The mean dry weight of the tops was greatest at 27°.

All Lovell-peach seedlings, Royal-apricot seedlings, and geranium cuttings in experiment 2 died at a soil temperature of 38° C. Top growth of the peaches was greatest at 31° and least at 10°; root growth was greatest at 10° and 17°, however, and least at 31°. The apricots also developed the largest tops at 31° and the smallest at 10°, but root growth was greatest at 17° and least at 10°. The largest geraniums, including both tops and roots, developed at 17°, though the plants at 10° were nearly as large; the smallest living plants developed at 31°.

Experiment 3, with seedlings of California pepper tree, involved two different levels of soil moisture. Definite amounts of water and air-dried soil were mixed together when the seedlings were potted, so that soil in the so-called "dry" series contained 6.5 per cent moisture, and that in the "wet" series, 17.7 per cent, on a dry-weight basis. A uniform distribution of moisture was not maintained in the "dry" series, however, because the small amounts of water applied to the surface at frequent intervals were retained in the upper two-thirds of the soil mass, and moisture in the bottom was probably reduced to the wilting range. This situation adversely affected the growth of the tops and the weight of the roots. A soil temperature of 10° C. retarded top growth, but the increments in terminal growth and in diameter of stem were greatest, and of similar magnitude, from 15° to 30°. The root systems were relatively small at 10° and at 30°; they were larger at intermediate levels, and were at their optimum at 15°.

In experiment 4, with rooted cuttings of Ragged Robin roses, there was

a definite trend in vegetative growth of the tops, from weakest growth, at 8° C., to strongest, at 28°. The largest number of feeder roots occurred at 23°. The terminal growth rate of casuarina seedlings (experiment 5) was greatly retarded at 7° and most rapid at 27°. Root development seemed best at 12° and 17°; at 27° it was comparatively slight.

Effect of Temperature on the Pathogen

The relation of temperature to the development of rhizomorphs of *Armillaria mellea*[3] was studied in the laboratory by growing the pathogen on 2 per cent potato-dextrose agar in large test tubes (Fig. 1). Twenty-one tubes

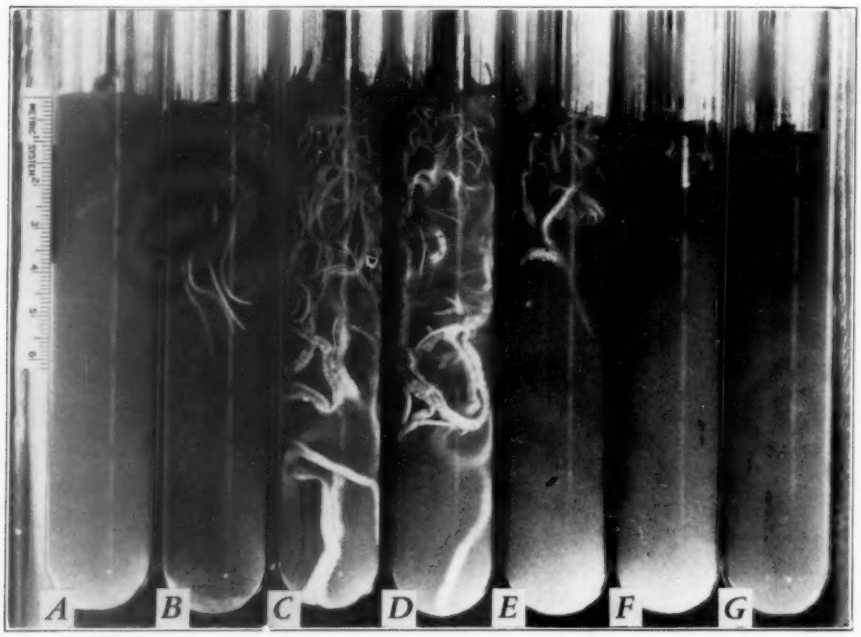


FIG. 1. Effect of temperature on the development of rhizomorphs of *Armillaria mellea* in 2 per cent potato-dextrose agar. Incubated 11 days at the following temperatures: A, 10°; B, 14.6°; C, 19.7°; D, 24°; E, 27.4°; F, 31°; G, 36° C.

containing young, well-established fungus colonies were divided into seven groups of 3 each and incubated 11 days at 10°, 14.6°, 19.7°, 24°, 27.4°, 31°, and 36° C., respectively. Judging from the relative size of the rhizomorphs and the rate at which they penetrated the media, the most favorable range of temperature was 19.7° to 24°. Rhizomorphs of normal appearance, but retarded in growth rate, developed also at 10°, 14.6°, and 27.4°. Very little growth occurred at 31°, and none at 36°. Reitsma (8), in Holland, found that *A. mellea* in peptone nutrient solution produced greater dry weight at 25° C. than at 15° to 19°, or at 10°.

In the inoculation experiments in the greenhouse, all containers except those of the controls were supplied with segments of freshly prepared roots

[3] Culture PB–41, isolated January 10, 1935, from citrus, Orange County, California.

TABLE 2.—*Effect of soil temperature on Armillaria root rot of citrus seedlings, experiment 1*

Soil temperature	Koethen sweet orange			Standard sour orange		
	Total plants tested	Plants attacked		Total plants tested	Plants attacked	
		Severely	Slightly		Severely	Slightly
°C.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
10	9	4	0	9	6	1
17	9	1	2	9	3	4
24	9	0	2	9	0	0
31	9	0	0	9	0	0
38	9	0	0	9	0	0

or stems containing viable mycelium of *Armillaria mellea.* This inoculum contained abundant food reserves—probably more than enough to maintain viability throughout the longest experiment (438 days). Some pieces of inoculum in the "dry" series of experiment 3 may have lost viability because of undue drying, but for the most part, viability was lost only at soil temperatures of 30° C., and above. *Armillaria* in all the experiments was viable in inoculum after 181 to 438 days at temperatures of 7° to 28°, inclusive. The largest development of rhizomorphs, both in number and in length,

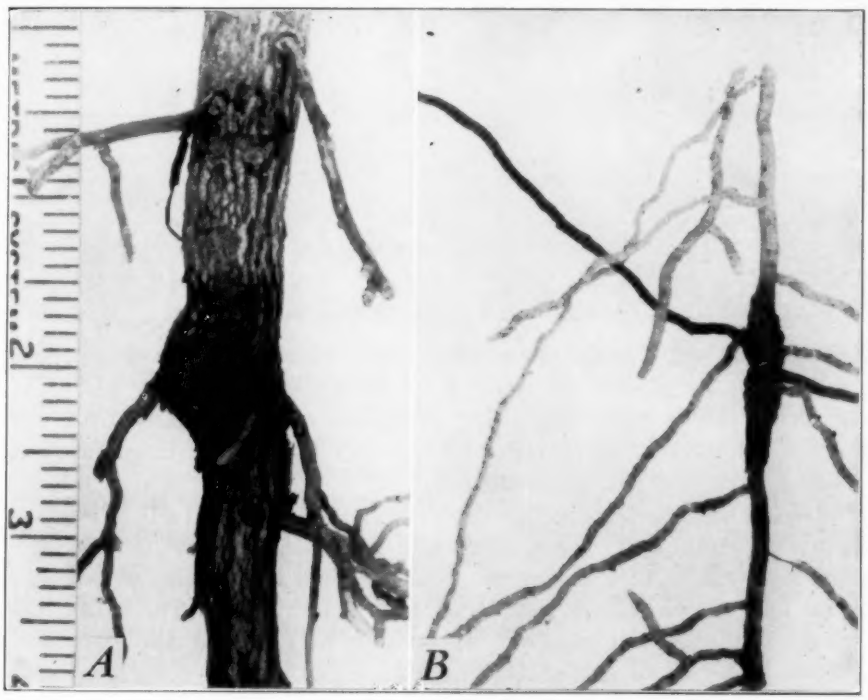


FIG 2. Roots of Koethen sweet orange, experiment 1, showing lesions of Armillaria root rot which developed at 10°: A, taproot; B, branched feeder root. Note attachment of dark fungus rhizomorphs to the lesions.

occurred at the lowest temperatures, and the least development, at the highest. This surprising tendency was observed in all the experiments.

Effect of Soil Temperature on Pathogenesis

None of the noninoculated plants in these experiments was infected with Armillaria root rot. Among the inoculated plants, infection was limited to those at soil temperatures in which the pathogen produced rhizomorphs. As demonstrated by Thomas (9), infection was initiated with the penetration by a branch of the parent rhizomorph directly through the sound, healthy periderm of the suscept. The method was similar in the different suscepts, regardless of their relative susceptibility. Citrus seedlings were considera-

TABLE 3.—*Effect of soil temperature on Armillaria root rot of Homosassa-sweet-orange and Sampson-tangelo seedlings, experiment 6*

Soil temperature	Culture B–700			Culture B–701		
	Total plants tested	Plants attacked		Total plants tested	Plants attacked	
		Severely	Slightly		Severely	Slightly
	Homosassa sweet orange					
°C.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
7	2	0	0	4	0	1
12	2	0	2	4	0	2
17	2	0	2	2	0	2
22	2	0	0	4	0	0
27	2	0	0	4	0	0
	Sampson tangelo					
°C.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
7	4	0	1	2	2	0
12	4	0	4	2	0	0
17	4	0	4	2	0	2
22	5	0	3	2	0	1
27	4	0	0	2	0	0

bly more resistant than the other plants that were tested. The citrus experiments, though comparatively long, were terminated before any plants had died or had shown marked secondary symptoms of Armillaria root rot. The effect of temperature on pathogenesis was therefore judged in citrus by the size and number of lesions produced.

In Koethen-sweet-orange seedlings, after 438 days, infection was comparatively severe at 10°, moderate at 17°, and slight at 24° C. (Table 2). Necrotic lesions on both the taproots and the small feeder roots were marked by the attachment of dark fungus rhizomorphs (Fig. 2). The lesions were swollen and contained considerable amounts of gum and also the typical white fungus fans of *Armillaria* in the bark and cambium. The presence of mycelium in the roots was also demonstrated by tissue cultures. Seedlings of Standard sour orange, under similar conditions, reacted much like those of sweet orange: the root rot was comparatively severe at 10° and moderate at 17° (Table 2).

In the citrus seedlings of experiment 6, the disease developed at temperatures of 7° to 22° C., but most rapidly at 12° and 17°. Homosassa-sweet-orange seedlings were not attacked at 22°, and were attacked only slightly at 7° (Table 3); but seedlings of Sampson tangelo were infected at all temperature levels, from 7° to 22°, inclusive, and appeared somewhat more susceptible than the sweet orange. The North Carolina strain of *Armillaria* (culture B–701) seemed to be nearly as pathogenic as the California strain (culture B–700) in this experiment, though in earlier, preliminary experiments with California pepper tree, poinsettia (*Poinsettia pulcherrima* Graham), and Royal apricot, it had seemed less pathogenic.

The relation of soil temperature to the development of Armillaria root rot was clearly demonstrated in experiment 2 (Table 4). Peaches, apricots, and geraniums, three very susceptible species, were infected at 10° C., but the disease progressed more rapidly at 17° and 24°, all the plants being

TABLE 4.—*Effect of soil temperature on Armillaria root rot of Lovell peach, Royal apricot, and geranium, experiment 2*

Soil temperature	Lovell peach[a]			Royal apricot			Geranium		
	Total plants tested	Plants attacked		Total plants tested	Plants attacked		Total plants tested	Plants attacked	
		Severely	Slightly		Severely	Slightly		Severely	Slightly
°C.	No.	No.	No.	No.	No.	No.	No.	No.	No.
10	9	5	4	3	1	1	3	2	1
17	9	9	0	3	3	0	3	3	0
24	9	9	0	3	3	0	3	3	0
31	9	0	0	3	0	0	3	0	0
38	9	0	0	3	0	0	3	0	0

[a] Delayed foliation affected 1 plant at 10°, 5 plants at 17°, and 3 plants at 24° C.

severely infected at these temperatures, and most of them dying during the experiment (Figs. 3 and 4). The plants at 31° and 38° escaped infection because the development of rhizomorphs was inhibited. At 38°, the plants died from excessive heat.

Large necrotic lesions developed in roots of both the peaches and the apricots, these lesions containing the characteristic mycelial fans of the pathogen and exuding copius amounts of gum (Fig. 5). Death occurred only when all roots were infected and the lesions had girdled the stem. The tops of the geraniums were not killed so easily: these plants, because of their succulent stems, remained alive even after the roots and the basal portion of the stem had died from effects of the disease (Fig. 6). A yellowish mottling, wilting, and necrosis of the leaf blades was noted (Fig. 6, B) during infection, and the older leaves dropped from the stems, leaving only a few of the youngest leaves at the apex. Even when the stem had been invaded and killed for several inches above the soil, the apical portion was alive and capable of generating a new root system.

Seedlings of California pepper tree, in experiment 3, had the following

numbers of infected plants (of the 7 inoculated plants in each lot) at the various soil temperatures:

Series	10°	15°	20°	25°	30° C.
"Dry"	2	4	4	4	0
"Wet"	5	7	6	6	0

Soil temperatures of 15° to 25° C., inclusive, were most favorable to the disease. Infection also occurred at 10°, but pathogenesis was slower. The

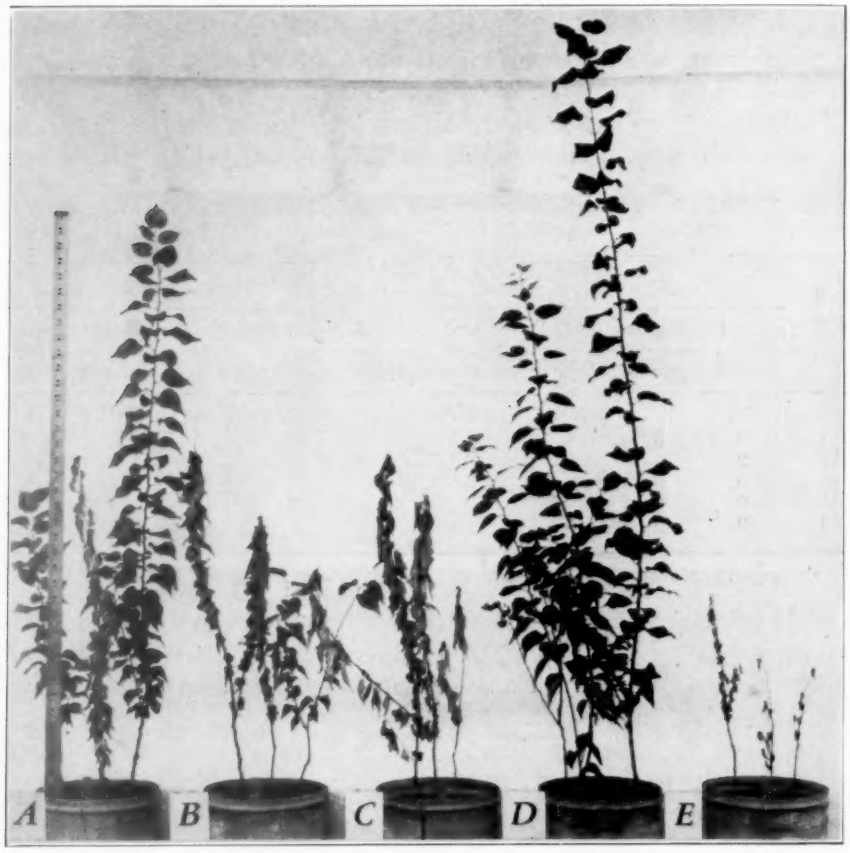


FIG. 3. Inoculated seedlings of Royal apricot, experiment 2, showing the effect of Armillaria root rot at different soil temperatures. A. Plants infected at 10° C., but injury moderate. B and C. Plants infected and dead at 17° and 24°, respectively. D. Large noninfected plants at 31°. E. Plants not infected, but killed by excessive heat at 38°.

plants at 30° were entirely healthy because growth of the pathogen had been inhibited. Secondary symptoms appeared first at 20° in diseased plants of the "wet" series, 67 days after inoculation, and at 25° in those of the "dry" series, 136 days after inoculation. Most root-rot lesions in the "dry" series were near the soil surface because dryness of the soil near the bottom of the containers had inhibited the growth of both roots and rhizomorphs. Soil

moisture, under these conditions, affected the rate of pathogenesis and also the percentage of infected plants. The symptoms observed during infection were rapid wilting and death following the complete girdling of the stem above the highest lateral root (Fig. 7).

In experiment 4, with Ragged Robin roses, the periods from inoculation until development of the first visible lesions, at the various temperatures, were as follows: 68 days at 18°, 77 days at 23°, 124 days at 13°, and 160 days at 8° C. Greatest injury from Armillaria root rot had occurred at 18°, when, after 181 days, the experiment was terminated: at this temperature 4 of the 6 inoculated plants were dead. Injury at 13° and at 23° was moderately severe, and at 8° it was moderate. All plants at 28° were strong and healthy. Of the 24 inoculated plants in the range 8° to 23°, inclusive, only

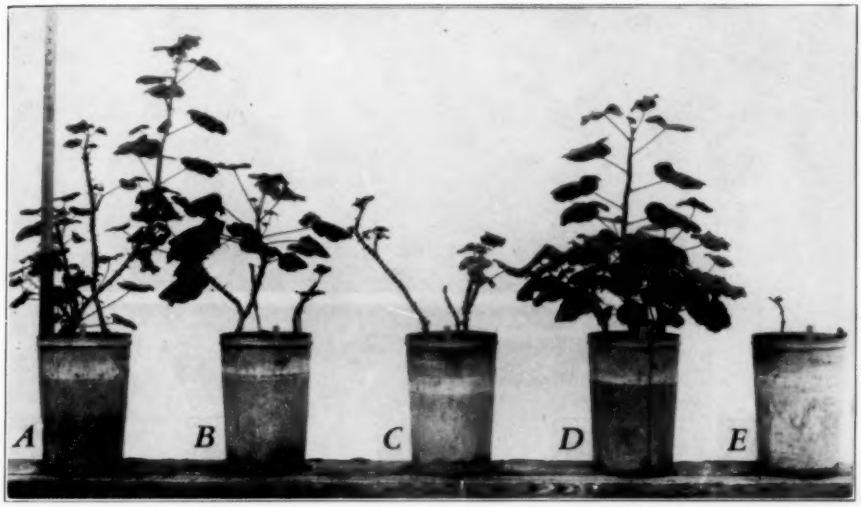


FIG. 4. Geraniums, experiment 2, showing the effect of different soil temperatures on the development of Armillaria root rot. A. Plants infected at 10° C., but injury moderate. B and C. Plants severely infected at 17° and 24°, respectively. D. Plants not infected at 31°. E. Plants not infected, but killed by excessive heat at 38°.

2 plants (at 23°) had escaped infection. Attack by rhizomorphs, which were most numerous at the lower temperatures, had produced many swollen and necrotic lesions on the roots and canes.

Casuarina seedlings, in experiment 5, showed the first visible symptoms of Armillaria root rot after 94 days at 22° C., after 164 days at 17°, and after 207 days at 12°. At the close of the experiment (after 324 days), there were dead plants at 12°, 17°, and 22°, but injury was most severe at 22°. Only slight to moderate injury was found at 7°. Of the 6 inoculated plants at each temperature level, all were infected at 7° and 12°, 5 were infected at 17°, 4 at 22°, and none at 27°. Typical primary symptoms were found, consisting of dark, necrotic, localized, swollen lesions in the stems and roots. The pseudosclerotium of the pathogen was visible as dark lines in longitudinal cracks in the bark. Some of the infected plants had survived in appar-

ently good condition by developing clusters of small lateral roots along the stem above the lesions.[4]

DISCUSSION

In the experiments reported in the present paper, pathogenesis occurred at soil temperatures of 7° to 25° C., inclusive. The maximum was between 25° and 27°; the minimum, probably slightly below 7°. The optimum, although broadly defined in most cases, varied from 10° to 25°. The reason

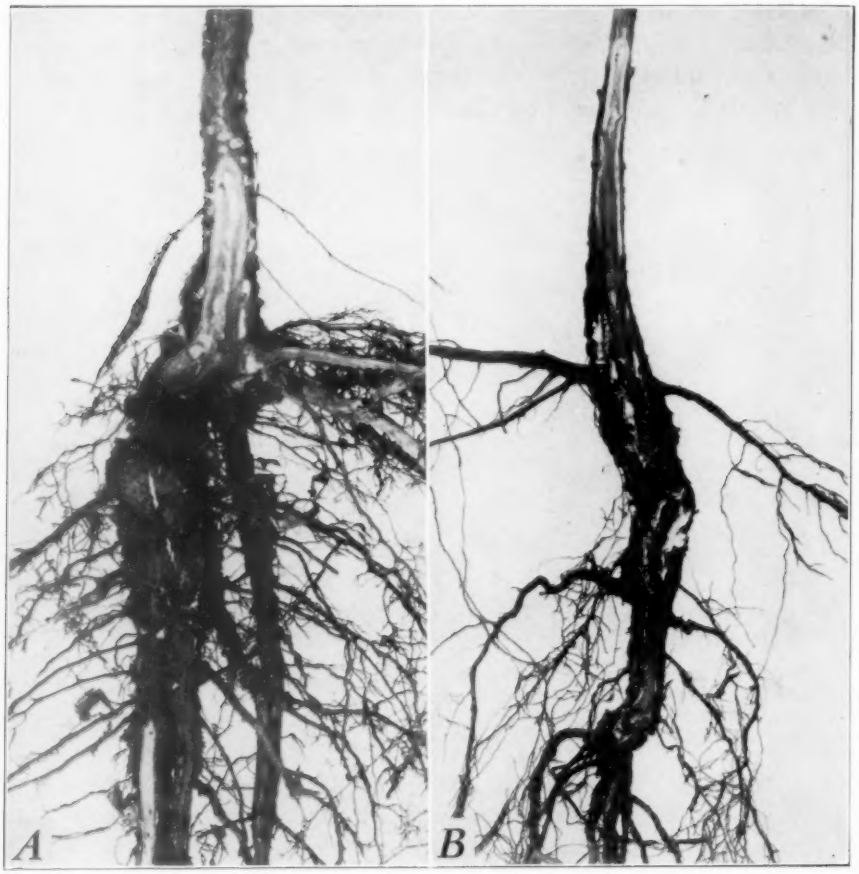


FIG. 5. Necrotic lesions of Armillaria root rot in roots of seedling plants of experiment 2: A, Lovell peach, at soil temperature of 24° C.; B, Royal apricot, at 17°.

for this variation was clarified somewhat when the temperature requirements of the different experimental plants were considered. The peach had the lowest optimum temperature range for root growth, and the rose the highest. When all nine species were arranged in this manner, they formed two groups (Fig. 8): A, those preferring the lower temperatures for root growth (10° to 17°); and B, those preferring the higher (17° to 31°). The first group included peach, casuarina, pepper tree, geranium, and apricot; the second,

[4] In experiment 5 other plants of casuarina had been inoculated in a similar manner with *Clitocybe tabescens* (Scop.) Bres., from Florida. The results were entirely negative.

sweet orange, sour orange, Sampson tangelo, and rose. The optimum range for pathogenesis in group A was relatively high (15° to 25°), while that in group B was relatively low (10° to 18°). Thus, an inverse relation existed between the optimum ranges for root growth and for pathogenesis. In other words, greatest resistance to Armillaria root rot was shown by all these plants at temperatures most favorable to root growth.

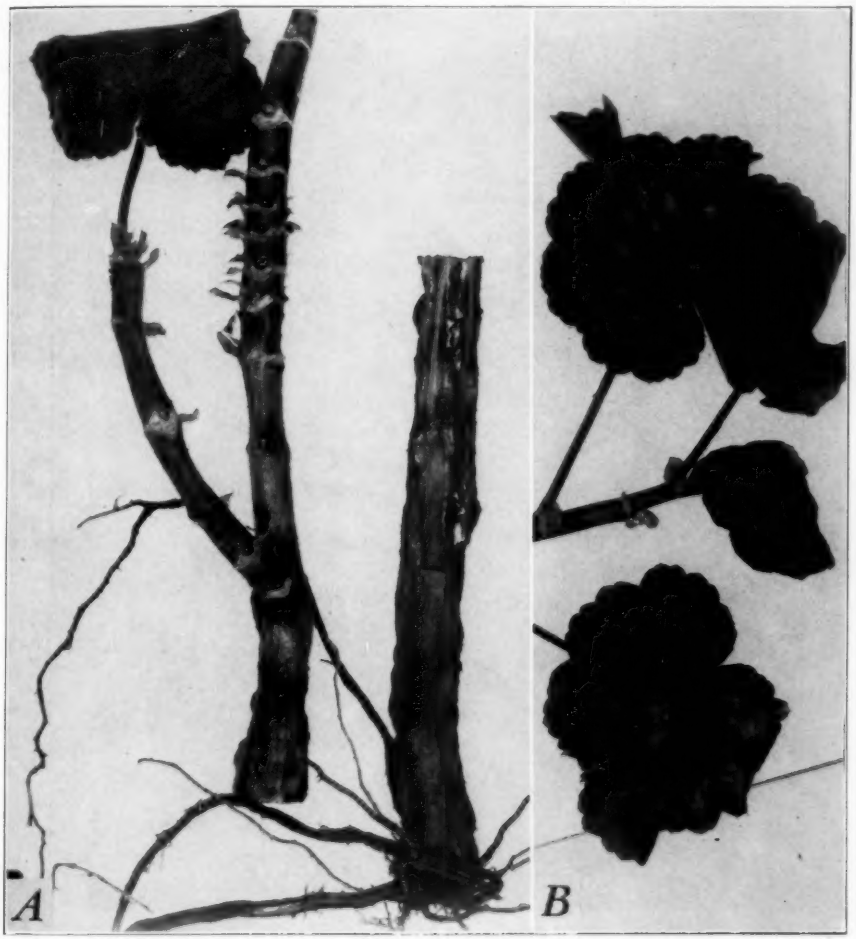


FIG. 6. Armillaria root rot of geranium, experiment 2. A. Severely infected plant at soil temperature of 17° C. B. Mottling, wilting, and necrosis of leaf blades at 10°.

The healthy condition of inoculated roots at soil-temperature levels above 25° C. was apparently due to freedom from attack. At 26° to 31°, plants in group B were healthy and also had maximum root growth; plants in group A were healthy, but root growth was poor in that range of temperature. Roots of peach, apricot, geranium, and citrus were killed at 38°. In these experiments the maximum temperature for root growth in citrus was approximately 37°, and that for peach, apricot, and geranium was approximately 34° (Fig. 8).

Freedom from attack at these higher temperatures may be explained by the response of the pathogen. In agar the rhizomorphs of *Armillaria* grew slowly at 27.4° and very little at 31° C. In woody inoculum, buried in soil, the mycelium was viable after 181 days at 28°, but nonviable after 240 days at 30°. Since very few if any rhizomorphs were produced in soil at temperatures above 25°, plants grown in this range escaped infection because the means of disease transmission were lacking.

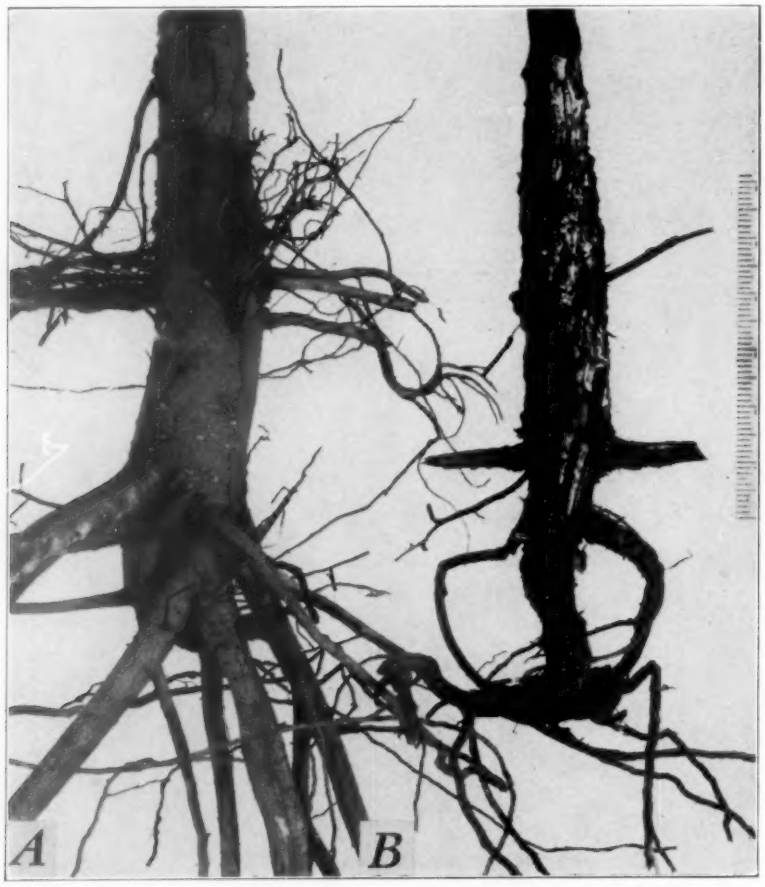


FIG. 7. Roots of California pepper-tree seedlings grown in soil at 15° C., experiment 3. A. Healthy control plant. B. Plant completely girdled and killed by Armillaria root rot. Some bark was removed to show the white mycelial fans of the pathogen.

The experiments on *Armillaria* indicate that the optimum temperatures for pathogenesis and for the growth of the pathogen are not always the same. A similar situation was discovered by Dickson (5) in regard to the seedling blights of wheat and corn, both of which are caused by *Gibberella saubinetii* (Mont.) Sacc. (optimum growth temperature, 24° to 28° C.). Wheat seedlings, growing better at low soil temperatures, were healthy at 8° but severely blighted at 16° to 28°. Corn seedlings, growing better at high soil temperatures, were severely blighted at 8° to 16°, but were healthy at 28°.

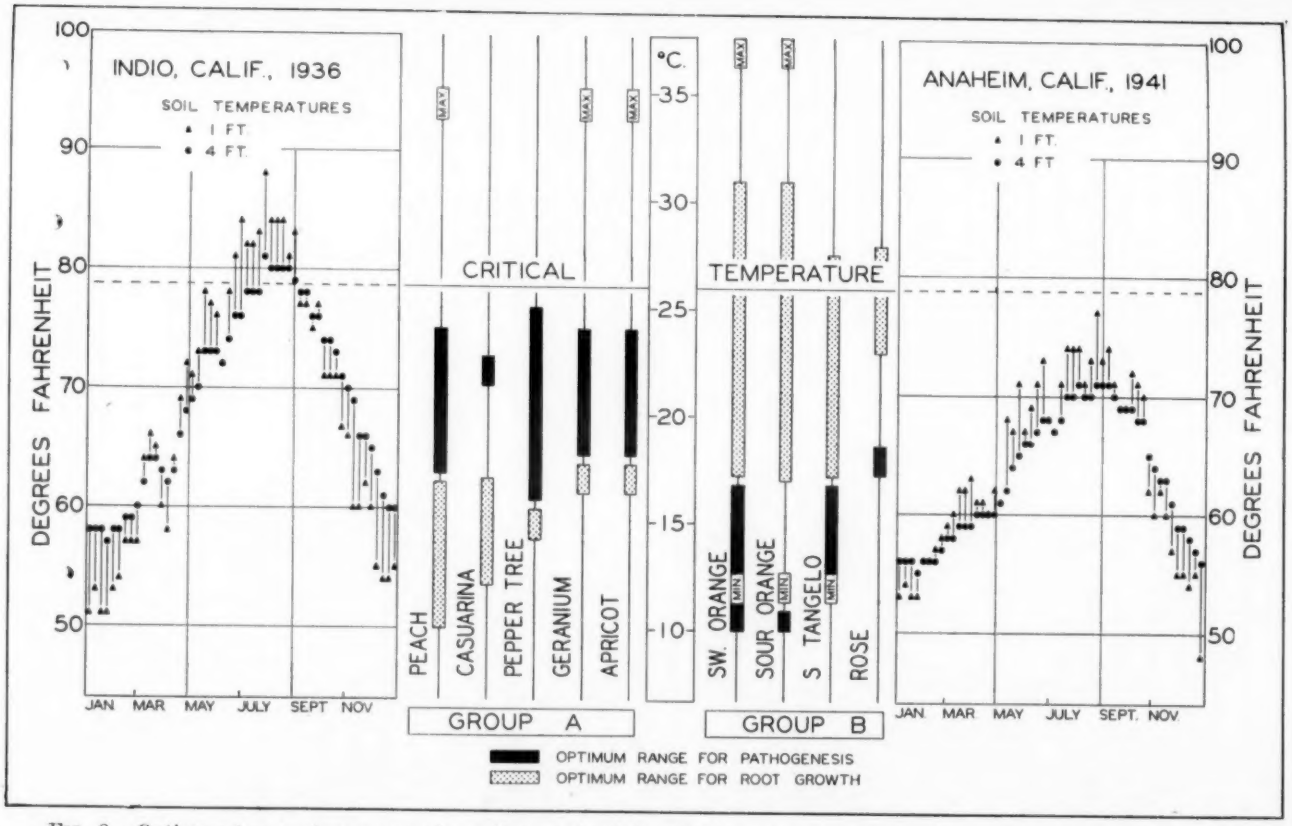


FIG. 8. Optimum temperature ranges for pathogenesis of Armillaria root rot and for root growth of nine species of plants used experimentally, compared with the march of soil temperatures (1- to 4-foot depths) at Indio, California, in 1936, and at Anaheim, California, in 1941. The minimum (min.) and maximum (max.) temperatures for root growth are indicated, where known. A critical temperature, above which pathogenesis did not occur, is tentatively indicated at 26° C. (78.8° F.).

The critical temperature for the seedling blight of wheat was 12°; that for seedling blight of corn was 20° to 24°. Dickson (5, p. 867) concluded that "the influence of environmental factors on the hosts seems to be the fundamental cause of susceptibility to the disease. The seedlings become susceptible when they are unable to respond favorably to the environment."

"Critical temperature," as used by Dickson (5), is taken to mean the boundary between two contiguous ranges of soil temperature, one in which pathogenesis occurs, and one in which it does not occur. Actually, in any suscept there are two critical temperatures for a disease, namely, the temperatures at the two extremes of the range for pathogenesis. For instance, the critical temperatures for Armillaria root rot in the California pepper tree are thought to be approximately 4° and 26° C., respectively.[5] The first is of minor importance because it probably lies below the minimum temperature for root growth; the second is important because the pepper tree is known to thrive in soil up to 30°. The disease may have slightly different critical temperatures in each of the nine species used in the present experiments, but in the absence of more exact information, the upper critical temperature is tentatively placed at 26° in all cases (Fig. 8).

From these considerations, relative susceptibility of the experimental plants should be defined in terms of soil temperature. All the plants were presumably immune above 26° C. Plants of group A were comparatively more susceptible at 22° than at 10°. Considering only the rate of pathogenesis in its optimum range in each suscept, the relative susceptibility in these plants may be described as follows: geranium and apricot, very susceptible; peach, pepper tree, rose, and casuarina, susceptible; and Sampson tangelo, sweet orange, and sour orange, moderately resistant.

There is an element of chance in the time and place of contact of roots and rhizomorphs. Under favorable conditions, the probability that infection will occur in a given time and space depends in part on the product of the respective concentrations of roots and rhizomorphs.

The longevity of an infected plant depends not only on its size and relative susceptibility, but also on the location of the lesions. A plant will die quickly if the main stem is girdled by lesions, but if the lesions are at the extremities of the roots, no secondary symptoms of the disease may appear for many months.

In southern California, Armillaria root rot is prevalent throughout the orange- and lemon-growing districts, which extend 10 to 55 miles inland from the coast. The disease has not been reported, however, from the hot desert valleys east of the San Jacinto mountains, where dates and grapefruit are grown. Studies have been made on the temperature of irrigated soils at Indio (4), in the desert region, and at Anaheim (3), in the coastal region (See fig. 8). At Indio, in 1936, the mean weekly temperatures of soil, at 1- to 4-foot depths, reached a maximum range of 81° to 88° F. (27.2° to 31.1° C.), while at Anaheim, in 1941, the maximum range at these depths was only 71° to 77° F. (21.7° to 25° C.). Since these data are typical of

[5] Rhizomorphs of *Armillaria mellea* grow very slowly in agar at 5° C.

their respective stations, soil in the principal root zone (1 to 4 feet) at Indio is largely above the upper critical temperature for Armillaria root rot about three months each year, while the soil of corresponding depth at Anaheim is usually not heated to this temperature. It seems probable, therefore, that excessive heat in the desert region is an important factor in preventing the development of this disease. The failure of certain artificial inoculation experiments at Riverside may also be attributed to high summer temperatures. Pathogenesis appears to be strongly influenced by seasonal fluctuations in soil temperature. From the data presented, pathogenesis in peach, casuarina, pepper tree, geranium, and apricot would be expected to develop most rapidly in the coastal region from spring to fall, while that in citrus and rose would develop most rapidly from late fall until spring.

SUMMARY

This paper reports a series of six soil-temperature experiments with Armillaria root rot on nine economic and ornamental species of plants, including Koethen and Homosassa sweet orange, Standard sour orange, Sampson tangelo, Lovell peach, Royal apricot, geranium, California pepper tree, Ragged Robin rose, and casuarina. The plants, potted in soil and well established, were inoculated with the pathogen, *Armillaria mellea* (Vahl) Quel., and grown 181 to 438 days, in the greenhouse, in five soil-temperature tanks. The water baths in these tanks were held at different, controlled temperatures ranging from approximately 7° to 38° C. Air temperatures in the greenhouse ranged mostly between 21° and 27°.

Rather wide differences were observed in the responses of noninoculated plants to soil temperature. Optimum temperatures for root growth in peach, casuarina, pepper tree, geranium, and apricot (group A) were 10° to 17° C., inclusive, while those for citrus and rose (group B) were 17° to 31°, inclusive.

In citrus, top growth was greatly retarded at soil temperatures of 10° to 12° C., the new leaves being small and very chlorotic; largest top growth occurred at 27° to 31°. The minimum temperature for root growth was 12°; the maximum, slightly below 38°.

All plants of peach, apricot, and geranium died at a soil temperature of 38° C. Top growth of peaches and apricots was greatest at a soil temperature of 31° and least at 10°; that of geraniums was greatest at 17° and least at 31°. Top growth of pepper trees was retarded at 10° but was vigorous and of nearly equal magnitude at 15° to 30°. The growth rate of roses increased from 8° to 28°. Top growth of casuarina was greatly retarded at 7° and most rapid at 27°.

The rhizomorphs of *Armillaria mellea* grew most rapidly in sterile, deep nutrient agar at 19.7° and 24° C. Rhizomorphs of normal appearance but retarded in growth rate developed at 10°, 14.6°, and 27.4°. Very little growth occurred at 31°, and none at 36°. Slight growth has also been found at 5°. The pathogen remained viable in inoculum at controlled tempera-

tures from 7° to 28°, inclusive, during 181 to 438 days. After these long periods, greatest development of rhizomorphs in nonsterile potting soil was evident at the lowest experimental temperatures. The apparent inconsistency of the effect of temperature on the development of rhizomorphs in agar and in soil is not explained.

Pathogenesis in test plants was observed at soil temperatures ranging from 7° to 25° C., inclusive. The maximum temperature was between 25° and 27°; the minimum, somewhat below 7°. The optimum range for pathogenesis in plants of group A was 15° to 25°, while that for plants of group B was 10° to 18°. Greatest resistance was shown by all plants at temperatures most favorable to root growth.

There are two critical temperatures for Armillaria root rot in any suscept: one at each extremity of the range for pathogenesis. The lower temperature is of minor importance, usually lying below the minimum for root growth, but the upper one is important, because many suscepts thrive at higher temperatures, at which they are free from attack. The disease may have slightly different critical temperatures in each of the nine species of plants here tested, but in the absence of more exact information, the upper critical temperature is tentatively placed at 26° C. in all cases.

In southern California the prevalence of Armillaria root rot throughout the coastal region, and its supposed absence in the inland desert areas, are apparently related to differences in soil temperature. Soil at Indio (desert region), at 1- to 4-foot depths, is largely above 26° C. for about three months every year; soil of corresponding depths at Anaheim (coastal region) seldom reaches this temperature. In the coastal region, pathogenesis in peach, casuarina, pepper tree, geranium, and apricot would be expected to develop most rapidly from spring to fall, while that in citrus and rose would develop most rapidly from late fall until spring.

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

LITERATURE CITED

1. BLISS, DONALD E. Relation of soil temperature to Armillaria root rot in California. (Abstr.) Phytopath. **31**: 3. 1941.
2. ———. Omphalia root rot of the date palm. Hilgardia **16**: 15–124. 1944.
3. ———. Air and soil temperatures in a California citrus orchard. Soil Sci. **58**: 259–274. 1944.
4. ———, D. C. MOORE, and C. E. BREAM. Air and soil temperatures in a California date garden. Soil Sci. **53**: 55–64. 1942.
5. DICKSON, JAMES G. Influence of soil temperature and moisture on the development of the seedling-blight of wheat and corn caused by *Gibberella saubinetii*. Jour. Agr. Res. [U.S.] **23**: 837–870. 1923.
6. GARRETT, S. D. Root disease fungi. 177 pp. Chronica Botanica Co. (Waltham, Mass.). 1944.
7. JONES, L. R., JAMES JOHNSON, and JAMES G. DICKSON. Wisconsin studies upon the relation of soil temperature to plant disease. Wisconsin Agr. Exp. Stat. Res. Bull. 71. 1926.
8. REITSMA, J. Studien über *Armillaria mellea* (Vahl.) Quél. Phytopath. Zeitschr. **4**: 461–522. 1932.
9. THOMAS, HAROLD E. Studies on *Armillaria mellea* (Vahl) Quel., infection, parasitism, and host resistance. Jour. Agr. Res. [U.S.] **48**: 187–218. 1934.

PHYTOPATHOLOGICAL NOTES

Bud Blight of Soybean Caused by the Tobacco Ring-Spot Virus.[1]—A disease of soybean caused by the tobacco ring-spot virus has been responsible for substantial losses in yield in the midwestern producing areas in recent years. It is not definitely known how long significant damage has been occurring but the losses in 1943 and 1944 exceeded all previous records and ranks this disease among the most destructive of the soybean.

Pierce[2] noted the destructive nature of this virus on soybean and certain other legumes, but did not observe its occurrence in nature. Samson[3] reported finding the disease in experimental plantings of vegetable soybeans in Indiana in 1941. Melhus[4] observed it in Iowa in 1942, and later Johnson[5] reported the disease on soybean in Ohio. It is likely that at that time, it was distributed extensively throughout the midwest in small amounts but had escaped detection.

In view of the increasing importance of this disease and the variety of symptoms which have come to be associated with it, it seemed desirable to report at this time the information which is available. This paper is concerned primarily with the identification and description. Control measures have not been found. Studies are underway at the U. S. Regional Soybean Laboratory to find means of combating this menacing disease.

Symptoms. The effect of the tobacco ring-spot virus on the soybean plant depends to a large extent upon the age of the plants at the time of infection. Like many virus diseases, the greatest effect on the host is in the young growing tissues.

Natural infection in the field is rarely observed before the plants are 7 to 8 weeks old. In Illinois, therefore, the disease appears in late July or early August. At this time, the symptoms consist of a characteristic curving of the terminal bud apparently due to unilateral elongation, as shown in figure 1, A. The young expanding leaves have a bronzed appearance and sometimes a few leaves drop from the plant. The pubescence on the young stem tip becomes more prominent than usual and may darken somewhat. Eventually the growing point becomes necrotic and assumes a brittleness which causes this portion to break off easily when touched. When the bud symptoms first appear, a careful search may disclose areas of reddish brown discoloration in the pith of the stems or branches, appearing first in the

[1] A publication by the U. S. Regional Soybean Laboratory, a cooperative organization participated in by the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration; and the Agricultural Experiment Stations of Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, Oklahoma, South Carolina, South Dakota, Tennessee, Texas, Virginia, and Wisconsin.

[2] Pierce, W. H. Viroses of the bean. Phytopath. **24**: 87–115. 1934.

[3] Samson, R. W. Tobacco ring spot on edible soybeans in Indiana in 1941. U. S. Dept. Agric., Plant Disease Reporter **26**: 382. 1942.

[4] Melhus, I. E. Soybean diseases in Iowa in 1942. U. S. Dept. Agric., Plant Disease Reporter **26**: 431–432. 1942.

[5] Johnson, Folke. Soybean streak in Ohio. U. S. Dept. Agric., Plant Disease Reporter **27**: 86–87. 1943.

vicinity of the nodes. Plants becoming infected at this stage seldom produce seed. However, they remain green, fail to ripen normally, and persist in the field until frost. The pith discoloration gradually increases until late in the season when the pith in the entire stem and branches may be discolored. Necrotic areas may extend to the exterior of the stem in some cases, particularly at the nodes. Streaking of the petioles and large leaf veins occasionally has been observed.

Symptoms resulting from infection near blossoming time when most of the terminal elongation has ceased present a somewhat different picture. The expanding tissues in the flowers and young pods are the main points

FIG. 1. A. Soybean plant infected with bud blight showing the characteristic curving of the terminal bud. B. Pod symptoms resulting from infection near blossoming time. Note distorted and shrunken pods. (Photograph B was furnished by courtesy of Dr. B. Koehler of the Illinois Agricultural Experiment Station.)

affected. This type of infection causes the greatest losses commercially since it is usually very extensive in the affected fields and causes considerable reduction in the amount of seed produced. A high percentage of the young pod clusters may wither and fall off within 10 days after infection. The most common symptom, however, is the dark blotching effect on the pods as illustrated in figure 1. B. Many of the pods that do not fall are shriveled and produce defective seed or none at all.

Soybeans that do not ripen normally often produce seed of very poor quality. Samples of seed from healthy and bud-blight infected plants are shown in figure 2. The physiologic conditions bringing about this poor seed quality may be brought on by other factors, as well as infection by this virus.

This condition, therefore, should not be invariably associated with the bud-blight disease.

Identification of the Virus. Symptoms produced on tobacco by this virus are characteristic of tobacco ring spot. Tobacco plants which have "recovered" from the initial stages of the disease and are symptomless do not again show symptoms when reinoculated with the soybean strains or strains from tobacco definitely known to be tobacco ring-spot virus. The converse is also true, that is, tobacco plants which have "recovered" from symptoms induced by known tobacco ring-spot virus, produce no further

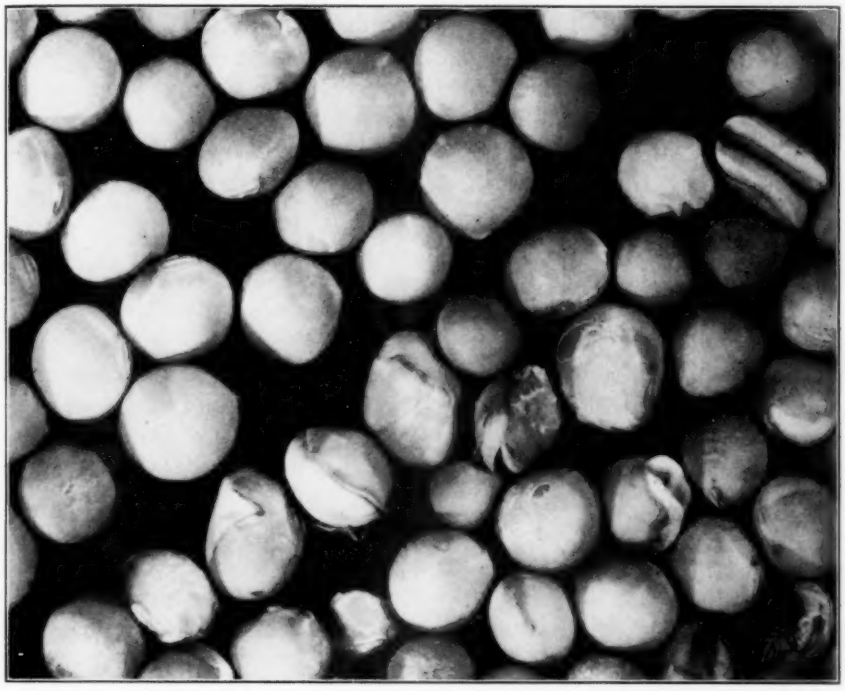


FIG. 2. Normal seed (left) of Bansei soybeans, and damaged seed (right) from artificially infected Bansei soybeans grown in adjacent rows in the field.

symptoms when inoculated with strains from soybean. Healthy plants of the same age become severely diseased upon inoculation. The soybean and tobacco strains were compared on garden bean (*Phaseolus vulgaris* L., var. Red Kidney). In both cases the plants were severely stunted and eventually succumbed to a progressive necrosis that usually started from the point of inoculation. Usually local necrotic lesions were produced upon the inoculated leaves. Symptoms on cucumber (*Cucumis sativus* L.) were indistinguishable with strains from soybean and tobacco. In both cases severe stunting and mottling continued indefinitely.

The thermal inactivation point corresponds exactly to that recognized for the tobacco ring-spot virus which is around 65° C. for 10 minutes. In

all the inactivation tests virus from soybean and known tobacco ring-spot virus were treated simultaneously.

Discussion. The occurrence of the tobacco ring-spot virus on soybean in the Midwest may help investigators to determine its mode of transmission. The agency accounting for its rapid spread through soybean fields is very likely one of the insects commonly associated with this crop. Typically the most damaging phase of the disease, *i.e.*, the pod spotting and blighting, occurs around the margins of the fields first and can be observed to progress inward as the season progresses. An intensive search is being made at the present time at this laboratory in an attempt to find an insect vector.

Summary. The symptoms produced on soybean by the tobacco ring-spot virus are described and illustrated. The identification of the virus by others was verified by thermal inactivation tests, "plant immunity" tests, and symptomology. The possibility of the occurrence of an insect vector is discussed.—WILLIAM B. ALLINGTON, Associate Pathologist, U. S. Regional Soybean Laboratory, Urbana, Illinois.

Two Aids for the Study of Potato-Late-Blight Epidemiology.[1]—An accurate measure of viable inoculum and an evaluation of environmental conditions that influence infection and the development of disease are essential to the study of plant disease epidemics.

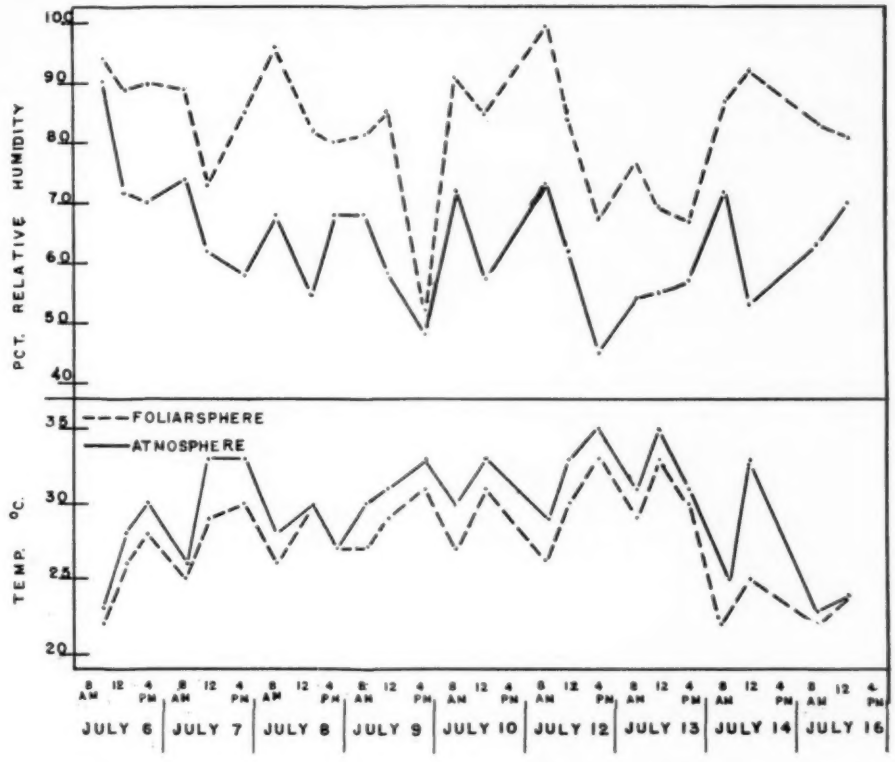
The presence in the air of sporangia of *Phytophthora infestans* can be determined fairly readily by exposing vaselined microscope slides which are subsequently examined with a microscope. Stem-rust inoculum has been measured successfully in this way, but the method has some disadvantages when used for late blight. First, the sporangia of late blight cannot readily be distinguished from those of several other downy mildews. Second, they are killed by short exposures to dry air, and, although the number of sporangia (in the absence of similar species) could be determined with a fair degree of accuracy with slides, assuming that slide exposures are an accurate criterion of the number falling on plants, the number capable of infection cannot be accurately measured.

In 1943 the writer exposed potted potato plants in several fields near St. Paul and Minneapolis, Minnesota. These plants had been raised in a greenhouse where fairly high temperatures as well as some protection from the outside air reduced the possibility of accidental infection. Check plants indicated that the possibility of accidental infection was zero. These plants were exposed in various locations, either overnight or for 4-hour periods during the day. At the end of the exposure period they were placed in portable moist chambers in an automobile and brought to the laboratory where they were incubated at about 70°F. and 100 per cent relative humidity. Control plants, directly from the greenhouse, were incubated at the same time and under the same conditions. None of the control plants became infected.

[1] Paper No. 2268, Journal Series, Minnesota Agricultural Experiment Station.

Late-blight lesions usually began to appear within 3 or 4 days after the plants were exposed. The lesions were counted, and their numbers, together with the numbers of sporangia found on slides exposed at the same time, were used to determine the "blight infection potential."

Having available an estimate of the viable inoculum at a given location, predictions regarding infection will depend upon an accurate knowledge of environmental conditions affecting germination and infection. Numerous attempts,[2, 3] have been made to correlate weather data with late blight epidemics, and some workers[4, 5] have found that mean temperature and precipi-



tation data are insufficient for the purpose. Total precipitation, if it comes as quick, heavy showers, may still be unfavorable for late blight if the intervening periods are dry. Prolonged periods of high humidity, however, may provide very favorable conditions for the disease even though the total precipitation be low.

It is suggested that ordinary weather observations may not be satisfac-

[2] Lutman, B. F. Twenty years' spraying for potato diseases. Potato diseases and weather. Vt. Agr. Exp. Stat. Bull. 159. 1911.

[3] Martin, W. H. Late blight of potatoes and the weather. N. J. Agr. Exp. Stat. Bull. 384. 1923.

[4] Gratz, L. O. Disease and climate as pertaining to the Florida and Maine potato sections. Phytopath. 20: 267–288. 1930.

[5] Orton, C. R. Meteorology and late blight of potatoes. (Abstr.) Phytopath. 6: 107. 1916.

tory for this purpose because such measurements are usually not made in the environment where infection occurs, *i.e.*, among the leaves of the potato plant. The observation that blight is often most severe in low spots in a field is evidence of the importance of temperature and humidity in limited environments rather than several feet from the ground and in open spaces where weather stations are usually located.

To compare the meteorological conditions in the atmosphere with those among the foliage of the plants (referred to as the "foliarsphere"), temperature and humidity were measured among the leaves of potato plants in the field and also at a point 5 feet above the plants. The relative humidity was measured by means of dew-point apparatus. The data (Fig. 1) show that although temperatures in the two locations differed but slightly, the differences in relative humidity were often very great. The differences were striking on July 8, 12, and 14.

There was 0.3 inch of rain on July 2 and again on July 12. Late blight was found in the field on July 7 and increased from then until July 19. Standard meteorological observations during this period indicate that the weather was unfavorable for blight development. On the other hand, the higher relative humidities in the foliarsphere indicate that among the leaves of the plant, moisture conditions favorable for fructification of the fungus and germination of inoculum probably obtained for sufficient periods for these processes to occur.

The data presented are preliminary in nature, and are not sufficient to show conditions at all hours of the day. Nevertheless they do illustrate the importance of the microclimate as a factor in the epidemiology of late blight and that potted potato plants exposed in fields when late blight inoculum is present may be used as indicators of the "blight infection potential."—W. D. THOMAS, JR., University Farm, Saint Paul, Minnesota.

A New Bean Mosaic in Idaho.[1]—In the summer of 1943 mosaic was reported by the field inspectors of the Idaho Crop Improvement Association in a field of Great Northern U.I. 15 beans grown near Buhl, Idaho. As this variety is resistant to common bean mosaic the records were carefully checked and it became evident that a new type of mosaic was present in this field. Inoculation experiments in the greenhouse have fully established this fact.

Some of the Great Northern U.I. 15 seed used to plant the field mentioned was secured and planted in the greenhouse. Twenty-five plants were grown from this seed and 2 developed mottling and curling of the leaves somewhat characteristic of common mosaic. The symptoms were not so severe as those which developed in Red Kidney plants grown from seed known to be infected with common mosaic. One rather marked characteristic of the new mosaic seems to be a tendency for the tip of the leaflets to curl downward. This tendency is more marked than in common mosaic. Otherwise the symptoms

[1] Published with the approval of the Director as Research Paper No. 245 of the Idaho Agricultural Experiment Station.

of the 2 mosaics are similar and it is impossible to distinguish between them on the basis of symptoms alone. Seed was secured by E. C. Blodgett, of the Federal Emergency Plant Disease Prevention, from the field of beans grown near Buhl, Idaho, where the disease was first noted. This seed was planted in the greenhouse and of 37 plants only 7 developed mosaic symptoms. Since Dr. Blodgett reported 85 per cent infection in the field, this is a surprisingly small amount of seed transmission.

Seed was collected from Great Northern U.I. 15 plants grown in the greenhouse and infected with the new bean virus when in the seedling stage. These plants all exhibited mosaic symptoms. This seed was planted in the field and a record taken of diseased plants which were produced. Of 113 plants grown from this seed, 68 developed marked symptoms of the disease and 45 were apparently healthy.

Mosaic-free seed of a number of bean varieties was planted in the greenhouse and the plants were inoculated with the virus from Great Northern U.I. 15 plants infected with the new mosaic. A modification of the method described by Pierce[2] was used. Diseased tissue was ground in a small food chopper, the plant juice was extracted and applied with cheesecloth to the primary leaves of the seedlings before the trifoliate leaves had developed. Carborundum powder was dusted lightly over the leaves before they were rubbed with the cheesecloth saturated with the plant juice. The plants were grown under artificial light in order to induce the mosaic symptoms. Red Kidney plants were inoculated with common bean-mosaic virus at the same time to check the method of inoculation. Every plant inoculated with the common mosaic virus developed mosaic symptoms. Ten plants of Great Northern U.I. 15 were inoculated with common bean-mosaic virus and no symptoms appeared on any. Noninoculated checks were grown under similar conditions.

As a result of these experiments in the University of Idaho greenhouses at Moscow and in the greenhouses at the field station at Twin Falls, Idaho, it has been shown that 2 strains of the common mosaic virus are present in Idaho. The University of Idaho Great Northern selections Num. 1, 56, 59, 81 and 123 are all resistant to the new strain of the virus as well as to the common mosaic virus previously used in testing these selections for resistance. Great Northern U.I. 15, Red Mexican U.I. 3, and Red Mexican U.I. 34 which were obtained by crossing the Great Northern variety with the Red Mexican variety are susceptible to the new strain but resistant to the common mosaic virus. Several pinto-type segregants from crosses between the Red Mexican selections resistant to common mosaic and the common Pinto beans all proved to be susceptible to the new virus strain although resistant to the common mosaic virus. The Michelite, Robust, Red Kidney, Bountiful, and Burtner are all susceptible to the new strain of bean virus, while Idaho Refugee and U.S. No. 5 are resistant.

[2] Pierce, W. H. The identification of certain viruses affecting leguminous plants. Jour. Agr. Res. [U.S.] 51: 1017–1039. 1925.

Burkholder[3] and Richards and Burkholder,[4] reported a new virus disease of beans in New York and state that Norida, Red Mexican U.I. 3 and Great Northern U.I. 15 are all susceptible to it. They also reported Great Northern U.I. 1 and Great Northern U.I. 59 as being immune.

Several lots of bean seed of varieties used by Richards and Burkholder were secured from B. Lorin Richards and their reactions to the new virus found in Idaho were compared with reactions to the one reported in New York. The results indicate that the 2 viruses are identical so far as host reaction of the varieties tested is concerned.

During the field inspections of bean fields in southern Idaho by the officials of the Idaho Crop Improvement Association in 1944 and again in 1945, the new strain of bean mosaic virus was found only in one locality on Great Northern U.I. 15. None of the fields had more than one per cent of the disease and as no tolerance for mosaic is allowed, and a good supply of certified seed is available, it is hoped that the disease may be kept under control until a resistant substitute can be developed.

Due to the fact that the Great Northern selections are all resistant and many hybrids have been developed in the bean-improvement program, it is hoped that from these we may be able to secure satisfactory segregants which will combine resistance to the curly-top and the new mosaic viruses.—LESLIE L. DEAN, Plant Pathologist of the Idaho Leafhopper Fund Administration, and C. W. HUNGERFORD, Idaho Agricultural Experiment Station, Moscow, Idaho.

[3] Burkholder, Walter H. New virus may threaten Robust pea-bean in New York. Farm Research **10**: 12, 16. 1944.

[4] Richards, B. Lorin, and Walter H. Burkholder. A new mosaic disease of beans. Phytopath. **33**: 1215–1216. 1943.